FOOD SAFETY AND FOOD SECURITY

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VALIDATION

Part II

Intra-Lab Validation

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What is Validation?

- What is Validation?

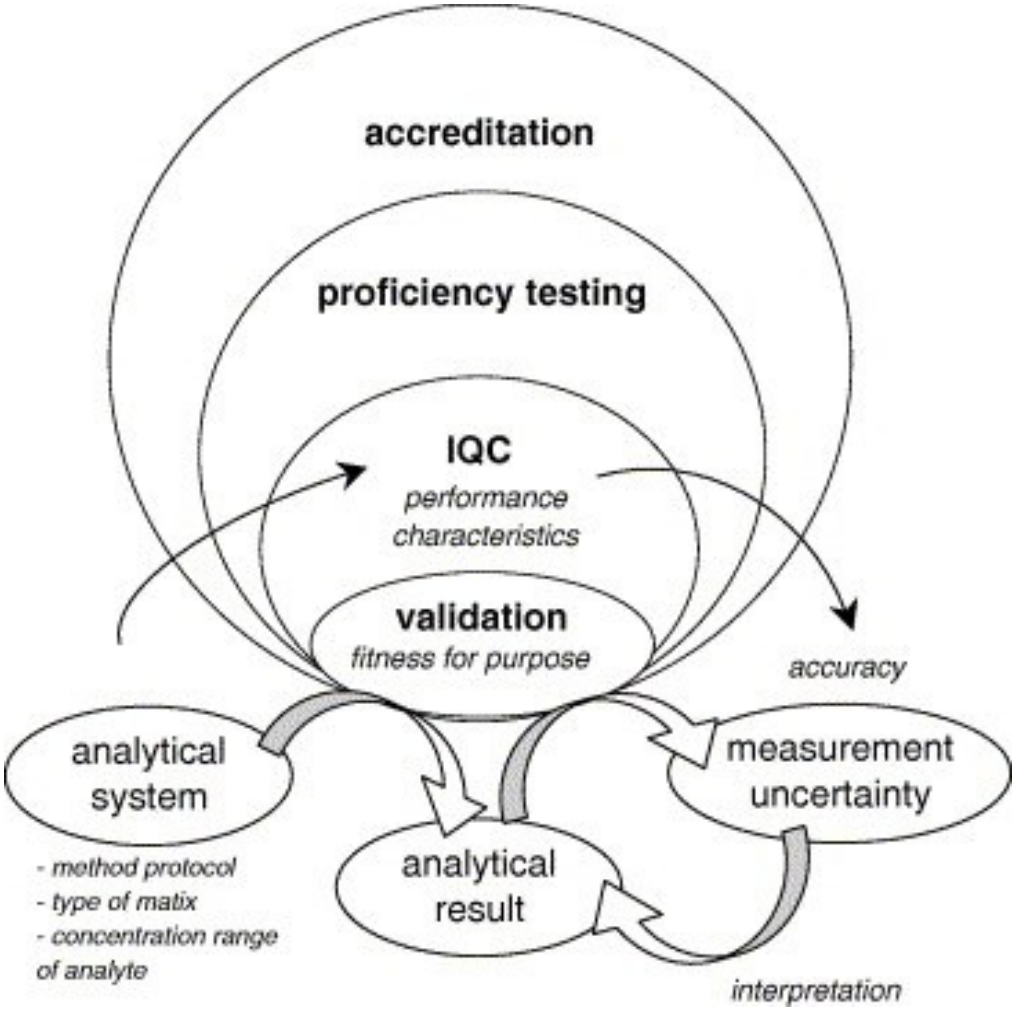
  Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled.

  ISO 9000

- Demonstrates that the analytical method is Fit For Purpose.

Why validate?

- Professional duty of the analytical chemist.
- Many decisions made based on the results of analytical measurements.
  - Health/safety
  - Fines or imprisonment
  - Valuing goods
- Provides laboratory knowledge e.g. critical steps in the analytical procedure.



Taverniers et al., TrAC 2004 23 535-552

Resources for Developing a Validation Procedure

- Legislation
  - **2002/657/EC** concerning the performance of analytical methods and the interpretation of results
  - **SANTE/11945/2015** Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.
  - The Water Framework Directive
  - ICH Guidelines
- Sector specific.
- May have different validation requirements.
- Sometimes even the terminology is different.
  - CCalpha and CCbeta.
  - Ruggedness versus robustness.

When to Validate

ISO/IEC 17025 5.4.5.2

- non-standard methods,
- laboratory-designed/developed methods
- standard methods used outside their intended scope
- amplifications and modifications of standard methods

When not to validate

- for standardised methods such as ISO, ASTM a full validation is not necessary
- need to *verify* the in-house performance of the method as detailed in ISO/IEC 17025 5.4.2
- *the laboratory shall confirm that it can properly operate standard methods before introducing the tests or calibrations*

Official Guidelines for Method Validation

- Eurachem → The Fitness of Purpose of Analytcial Methods
- ICH (International Conference for Harmonization) → Registration of Pharmaceutical
- AOAC (Association of Analytical Chemistry)
- IUPAC (International Union of Pure and Applied Chemistry)

  } Single Laboratory method validation

- EMA (European Medicines Agency)
- FDA (Food and Drug Administration)

  } Biomedical Methods

- SANCO (Directorate-General of Food and Safety) → Guidelines for Pesticides residues analysis in feed and food

Quantitative Analytical Method

Criteria required

- **Precision**
  - Repeatability
  - Within-lab reproducibility
- **Bias**
  - Matrix/substrate effects
  - Specificity
- **Working range**
  - Limit of detection/sensitivity
  - Linearity
- **Robustness**
  - Environmental susceptibility

Eurachem guide: Terminology in analytical measurement – Introduction to VIM 3 (2011) available from www.eurachem.org.

Quantitative Analytical Method

Accuracy:

Exactness of an analytical method

1.Precision:

The closeness of agreement between independent test results obtained under specific conditions → Random error

- Repeatability:
- Intermediate precision
- Reproducibility

Measure as Relative standard deviation (RSD %); (n= 6/10)

2. Trueness/correctness:

The closeness of agreement between the expected test result and the accepted reference value → Systematic error

Measure as Bias

$b = x - x_{ref}$

NO precise
NO correct

Precise
NO correct

NO precise
correct

ACCURATE:
Precise and correct

Quantitative Analytical Method

Accuracy:

Exactness of an analytical method

1.Precision:

The closeness of agreement between independent test results obtained under specific conditions ⟹ Random error

- **Repeatability**: same method, identical test, same laboratory, same operator, same equipment, short interval of time.
- **Intermediate precision**: same method, identical test, same laboratory, *BUT different* operator, equipment, longer interval of time.
- **Reproducibility**: same method, identical test, BUT different laboratory, operator, equipment

Measured as:

- Standard deviation (SD or s) or
- Relative standard deviation (RSD or s_{rel})
- Coefficient of variation (CV %) or RSD %
- Repeatability limit (r) = 2.83 x SD_r or reproducibility limit (R)= 2.83 x SD_R
- Confidence interval (CI) = x ± C

$$C = \frac{s\,x\,t}{\sqrt{n}}$$

Quantitative Analytical Method

Accuracy:

1.Precision:

Calculated repeatability, intermediate precision and reproducibility values can be compared with those of existing methods. If there are no methods with which to compare the precision parameters, theoretical relative reproducibility and repeatability standard deviations can be calculated from the Horwitz equation or from the values according to the AOAC Peer Verified Programme

Table 4. Horwitz function as an empirical relationship between the precision of an analytical method and the concentration of the analyte regardless of the nature of the analyte, matrix and the method used. Acceptable RSD_R and RSD_r values according to [27] and to AOAC International [8,14] (PVM = Peer Verified Methods (Program))

Analyte %	Analyte ratio	Unit	Horwitz %RSD	AOAC PVM %RSD
100	1	100%	**2**	1.3
10	1.00E−01	10%	**2.8**	2.8
1	1.00E−02	1%	**4**	2.7
0.1	1.00E−03	0.10%	**5.7**	3.7
0.01	1.00E−04	100 ppm	**8**	5.3
0.001	1.00E−05	10 ppm	**11.3**	7.3
0.0001	1.00E−06	1 ppm	**16**	11
0.00001	1.00E−07	100 ppb	**22.6**	15
0.000001	1.00E−08	10 ppb	**32**	21
0.0000001	1.00E−09	1 ppb	**45.3**	30

Horwitz= $2e^{(1-0.5logC)}$

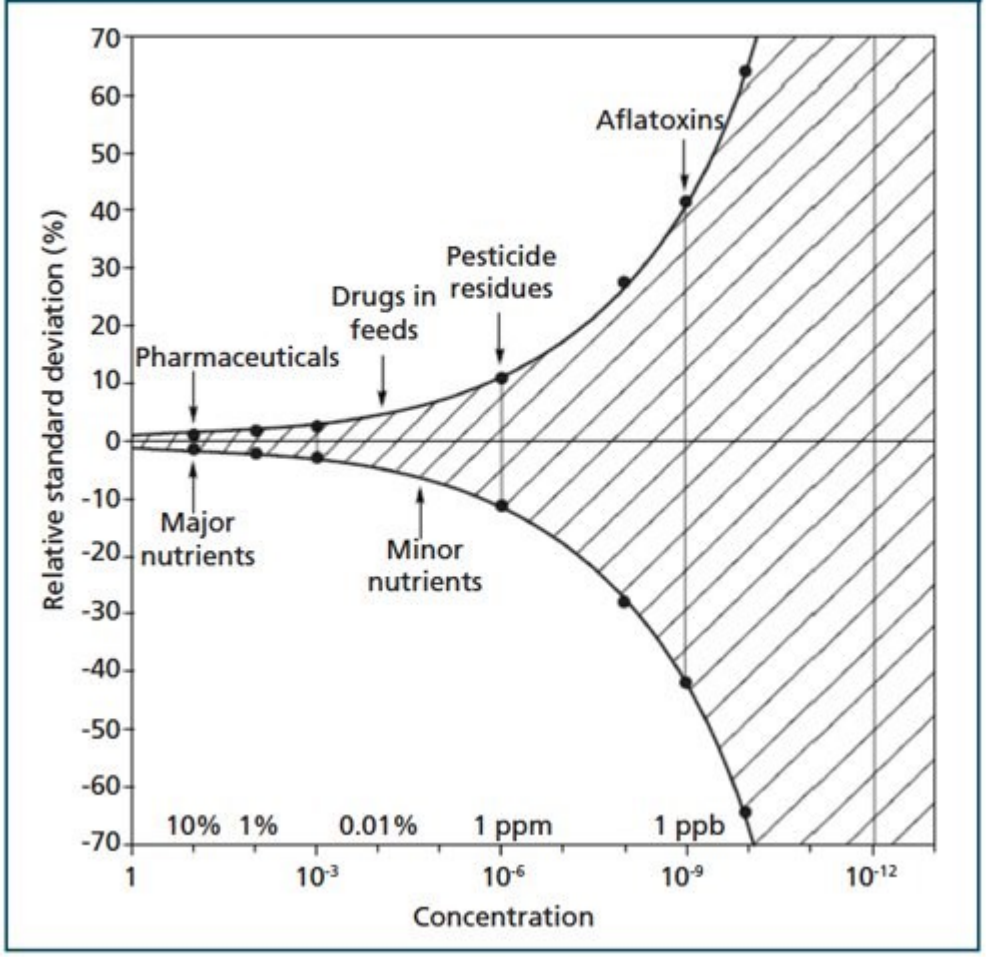
Trends in Analytical Chemistry, Vol. 23, No. 8, 2004 - Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance

Quantitative Analytical Method

Accuracy:

1.Precision:

Figure 1: The Horwitz "trumpet" displaying the inverse relationship between analyte concentration and relative standard deviation of sampling. (Adapted from reference 2.)



Quantitative Analytical Method

Accuracy:

Exactness of an analytical method

1.Precision:

The closeness of agreement between independent test results obtained under specific conditions ⟹ Random error

- **Repeatability**: same method, identical test, same laboratory, same operator, same equipment, short interval of time.
- **Intermediate precision**: same method, identical test, same laboratory, *BUT different* operator, equipment, longer interval of time.
- **Reproducibility**: same method, identical test, BUT different laboratory,operator, equipment

- ✓ 6-15 replicates for each material
- ✓ 3 concentration levels, minimum of 3 replicates per level

Quantitative Analytical Method

Accuracy:

Exactness of an analytical method

2. Trueness/correctness:

The closeness of agreement between the expected test result and the accepted reference value → Systematic error

Measured as:

- Bias (b= $x-x_{ref}$)
- Bias % (b =$100*[(x-x_{ref})/x_{ref}]$
- Relative % recovery ($R\% = 100*x/x_{ref}$)
- Z-score

$$z = \frac{X_{found} - X_{certified}}{\sqrt{\frac{SD_{found}}{n_{found}} + \left(\frac{SD_{certified}}{n_{certified}}\right)^2}}$$

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Quantitative Analytical Method

Accuracy:

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Using :
- **CRM:** preferred expression z-score
- **Spiked sample:** preferred expression R% or b(%)
- **Compared with a validated reference method:**

NO precise
NO correct

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Validation of the method : the use of CRM : an example

Standard or Certified Reference Material (SRM or CRM)

SRMs can be purchased from a number of governmental and industrial sources.

For example, the National Institute of Standards and Technology (NIST) offers over 1300 standard reference materials including rocks and minerals, gas mixtures, glasses, hydrocarbon mixtures, polymers, urban dusts, rainwaters, and river sediments.

The concentration of one or more of the components in these materials has been determined in one of three ways:

(1) By analysis with a previously validated reference method,

(2) by analysis by two or more independent, reliable measurement methods, or

(3) by analysis by a network of cooperating laboratories that are technically competent and thoroughly knowledgeable with the material being tested.

Alternative when not available: fortified samples

Validation of the method : the use of CRM : an example

Comparison of measurement results with the certified value has been done following a procedure :

1- calculate Δm, the difference between the certified value (C_{CRM}) and the mean measured value (C_m):

$$\Delta_m = |C_m - C_{CRM}|$$

There is no significant difference between the "experimental" result and the certified value if:

$$\Delta_m \leq U_\Delta$$

Difference between the averages < extended uncertainty

Validation of the method Use of CRM : an example

2- Calculate the combined uncertainty of result and certified value (u_Δ) is given by adding the uncertainty of the measurement result (u_m) and the uncertainty of the certified value (u_{CRM}). Uncertainties are expressed in standard deviation but only the variances are additive.

$$\mathbf{u_\Delta} = \sqrt{u_m^2 + u_{CRM}^2}$$

$\mathbf{u_{CRM}}$ is obtained by dividing the estimated expanded uncertainty by the coverage factor (k). A coverage factor is a security factor associated to the uncertainty in order to get into an interval of a given level of confidence.
k=2 defines an interval having a level of confidence of approximately **95%**.

$\mathbf{u_m}$ is obtained by dividing the **SD** (s_m) by the square root of the number of measurements (n)

$$\mathbf{u_m} = {}^{s_m}\!/_{\sqrt{n}}$$

The **expanded uncertainty** (**U**Δ) is given by multiplication of uΔ by a coverage factor (k, usually equal to 2):

$$\mathbf{U_\Delta = 2 * u_\Delta}$$

Validation of the method Use of CRM : an example

Certified value of PCB 52 in a fat animal = **12.9 ± 0.9** µg / Kg

A correction factor (×2) was used for define uncertainty

$$u_{crm} = 0.9/2 = 0.45 \text{ µg/Kg}$$

Laboratory measurements (N=6) : **14.3 ± 1.8** µg/Kg

$$u_m = {}^{s_m}/_{\sqrt{n}} = {}^{1.8}/_{\sqrt{6}} = 0.74 \text{ µg/Kg}$$

$$\Delta_m = |C_m - C_{CRM}| = |14.3 - 12.9| = 1.4 \text{ µg/Kg}$$

$$u_\Delta = \sqrt{u_m^2 + u_{CRM}^2} = \sqrt{0.74^2 + 0.45^2} = 0.87 \text{ µg/Kg}$$

$$U_\Delta = 2 * u_\Delta = 1.74 \text{ µg/Kg}$$

$$\Delta_m \leq U_\Delta$$

No significant difference

Quantitative Analytical Method

Accuracy:

Exactness of an analytical method

2. Trueness/correctness:

The closeness of agreement between the expected test result and the accepted reference value → Systematic error

Minimum trueness of quantitative methods

Mass fraction	Range
≤ 1 µg/kg	– 50 % to + 20 %
> 1 µg/kg to 10 µg/kg	– 30 % to + 10 %
≥ 10 µg/kg	– 20 % to + 10 %

From Decision 2002/657/EC

NO precise
NO correct

Precise
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Quantitative Analytical Method

Accuracy:

Exactness of an analytical method

2. Trueness/correctness:

The closeness of agreement between the expected test result and the accepted reference value → Systematic error

Analyte%	Analyte ratio	Unit	Mean recovery (%)
100	1	100%	**98–102**
10	1.00E – 01	10%	**98–102**
1	1.00E – 02	1%	**97–103**
0.1	1.00E – 03	0.10%	**95–105**
0.01	1.00E – 04	100 ppm	**90–107**
0.001	1.00E – 05	10 ppm	**80–110**
0.0001	1.00E – 06	1 ppm	**80–110**
0.00001	1.00E – 07	100 ppb	**80–110**
0.000001	1.00E – 08	10 ppb	**60–115**
0.0000001	1.00E – 09	1 ppb	**40–120**

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Confidence interval

Calculation of the **standard deviation** for a set of data provides an indication of the precision inherent in a particular procedure or analysis.

But unless there is a large amount of data, it does not by itself give any information about how close the experimentally determined mean x might be to the true mean value μ.

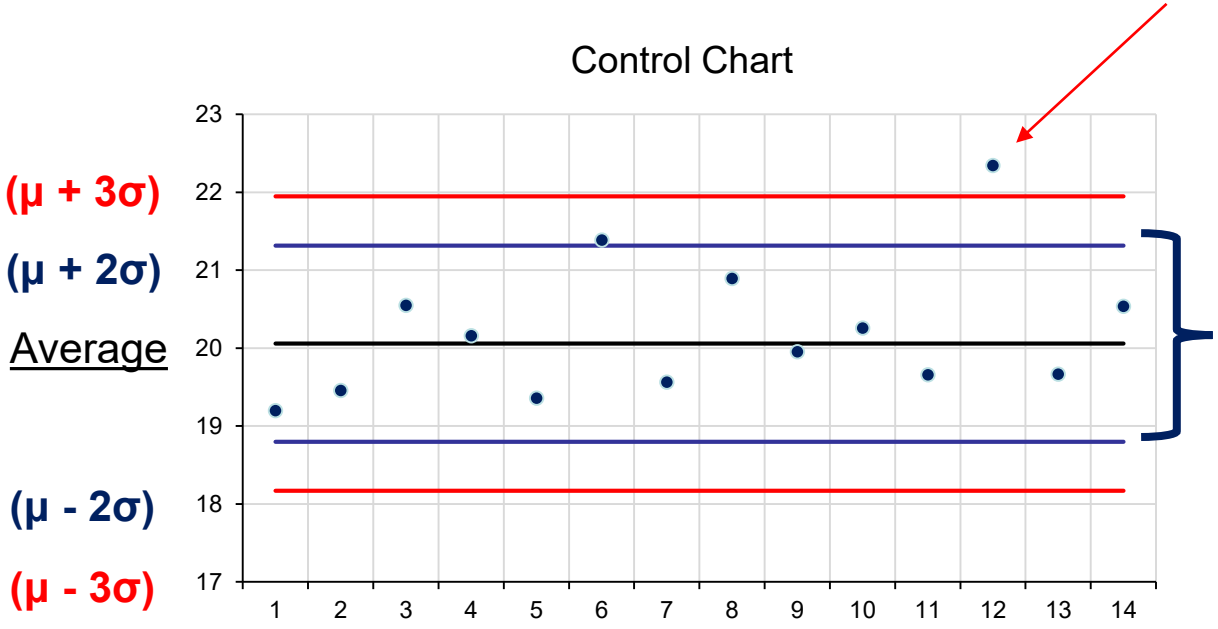
Statistical theory, though, allows us to estimate the range within which the true value might fall, within a given probability, defined by the experimental mean and the standard deviation.

$$\text{Confidence limit} = \bar{x} \pm \frac{ts}{\sqrt{N}}$$

Confidence interval: the Control Chart

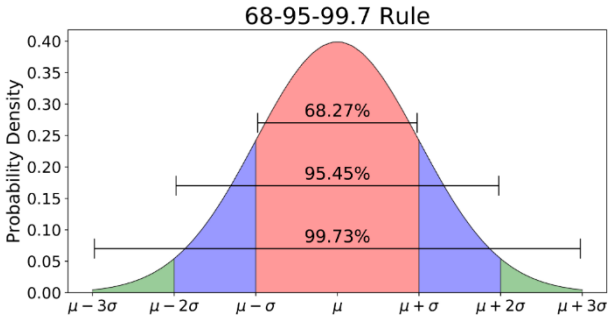
is a visual representation of confidence intervals for a Gaussian distribution.

ISO Standard 8258:1991 provides for various scenarios that constitute an anomaly.



Warning criteria

μ ± 2σ : 95.5% of the data are within this range



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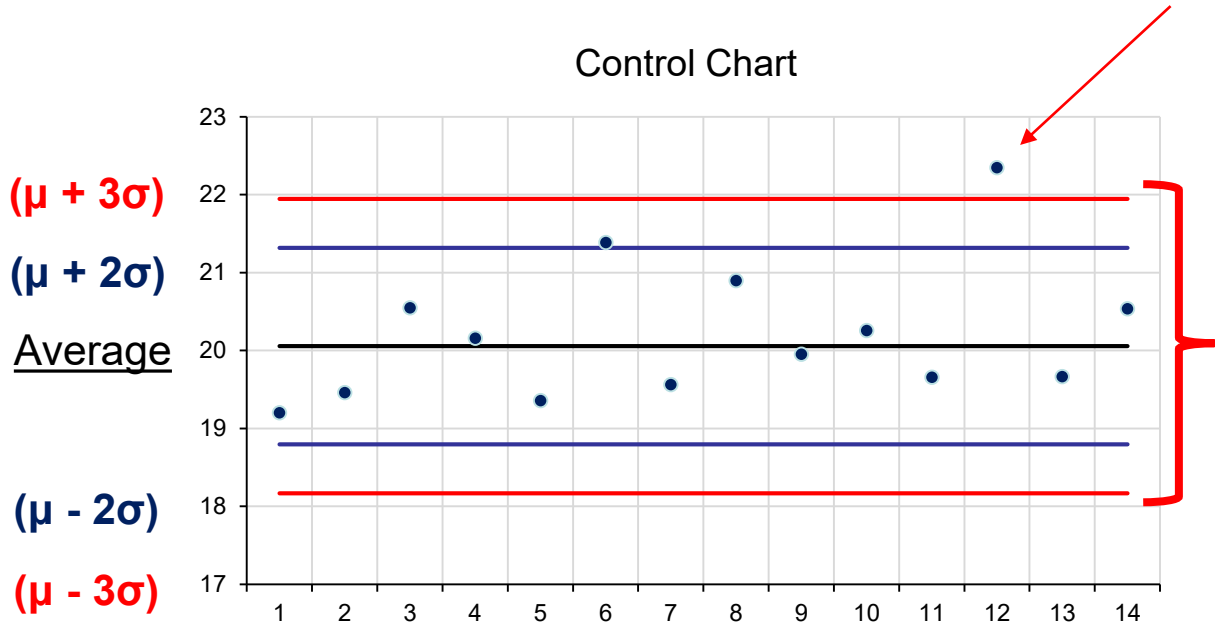
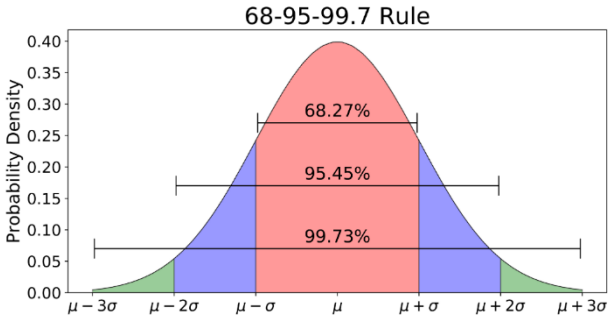


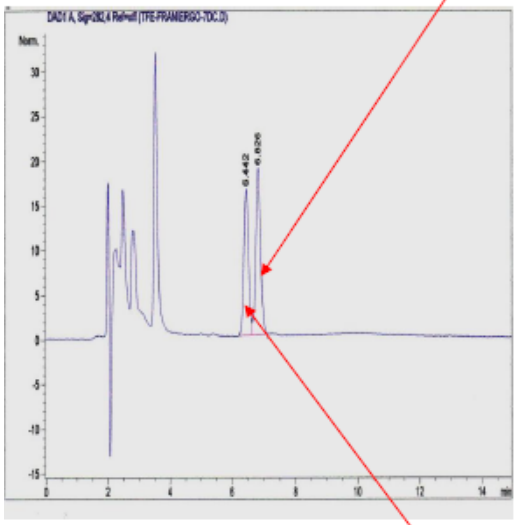
Alert criteria

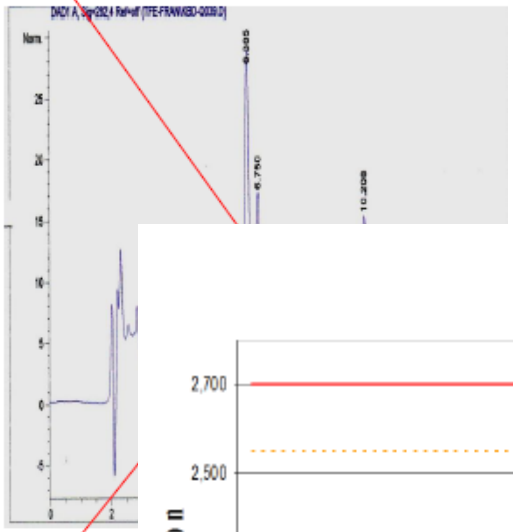
μ ± 3 σ : 99.7% of the data are within this range



An example of a quality control chart adapted to HPLC analysis.

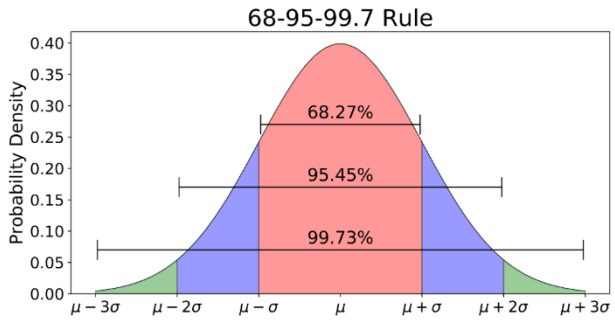
7-déhydrocholesterol

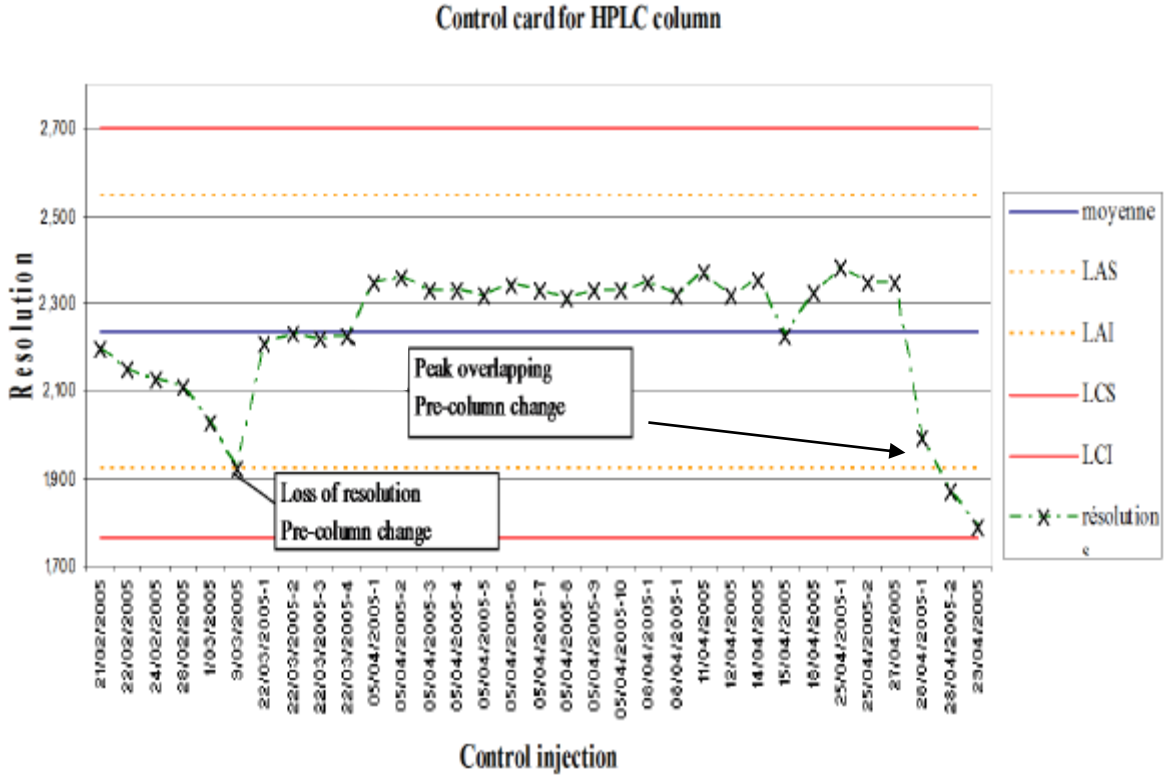




References

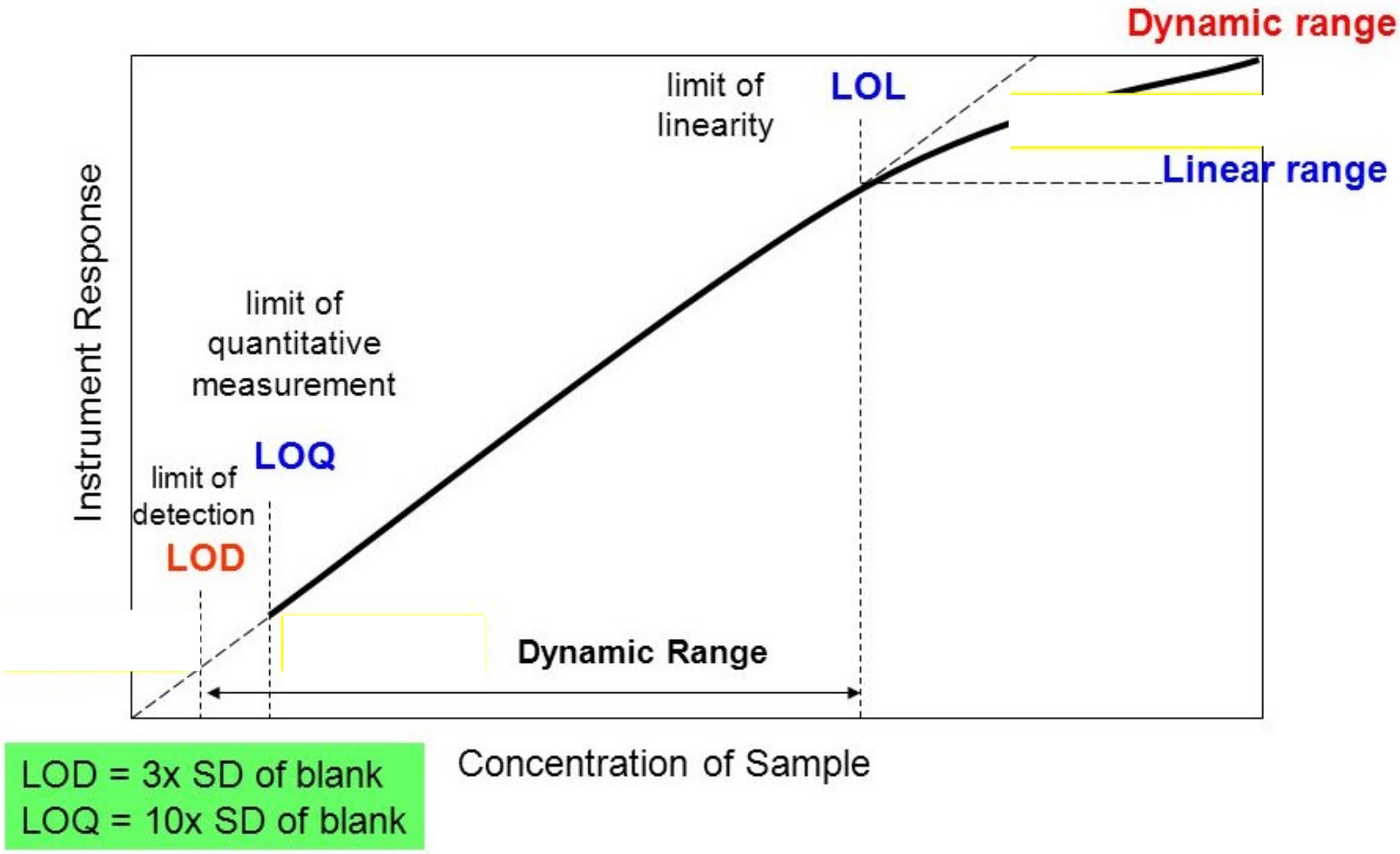
Ergosterol non volatile BIOMARKI





Quantitative Analytical Method

Linearity



Adapted from Figure 1-7 in Skoog, D.A., *et al.* (1998) *Principles of Instrumental Analysis* (5th Edition). Thomson Learning, Inc.

Quantitative Analytical Method

Linearity: defined as the ability of the method to obtain test results proportional to the concentration of analytes within a given range

Quantification strategy in instrumental analytical chemistry

- **External standard**
- **Internal standard**
- **Matrix-matched**
- **Standard addition**

→

- **Calibration**
- **Response factor**

Quantification strategy in instrumental analytical chemistry

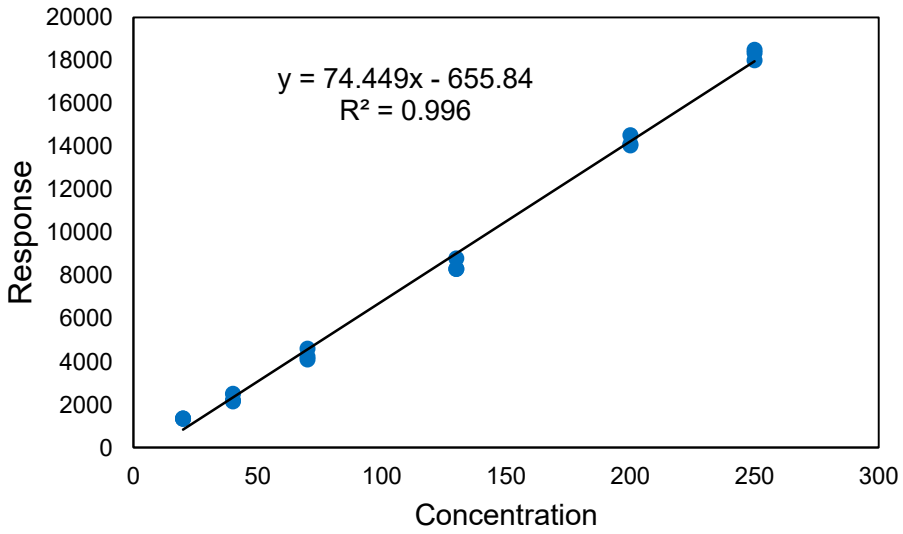
- **External standard Calibration**

  - Standard solution containing compounds to be quantified

  - It can be performed in a single point, assuming linearity

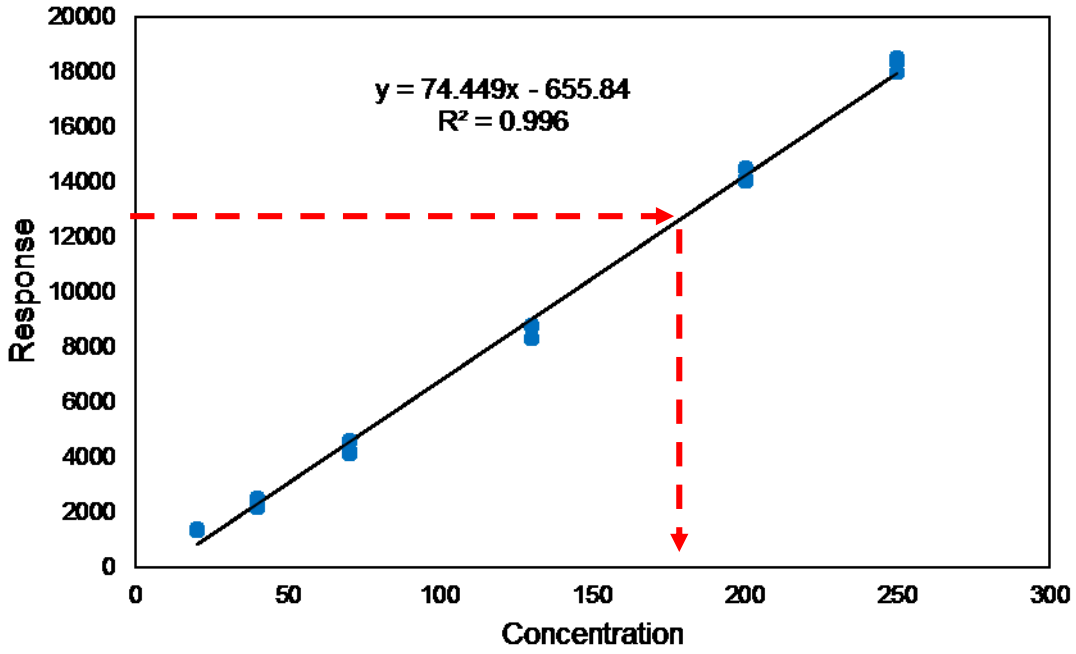
$$C_x = \frac{A_x}{A_{std}} \times C_{std}$$

  - Calibration and linearity assessment



Quantification strategy in instrumental analytical chemistry

- **External standard**
  - Calibration and linearity assessment
    - ✓ Different Guidelines different requirements
    - ✓ 5-6 concentration levels are generally accepted, at least 3 replicates per level



Quantification strategy in instrumental analytical chemistry

- **External standard**

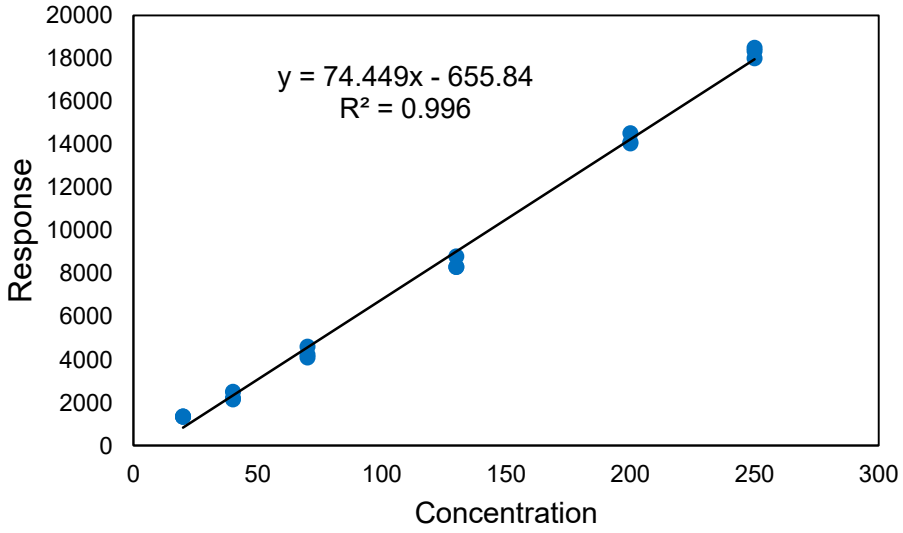
Advantages

- It is fairly simple

BUT

Disdvantages

- Assume quantitative transfer at each step. No compensation for losses.
- No compensation for **matrix effect**



Quantification strategy in instrumental analytical chemistry

- **Internal standard**

A fixed quantity of a standard is added at earlier as possible in the analytical procedure.

Assumption: any changes in the injected amount of the component(s) of interest, e.g. due to sample preparation losses, correspond to equal changes in the injected amount of the internal standard component.

Critical point: selection of internal standard(s)

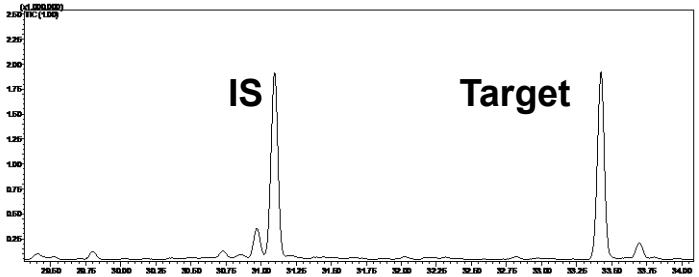
Quantification strategy in instrumental analytical chemistry

- **Internal standard**

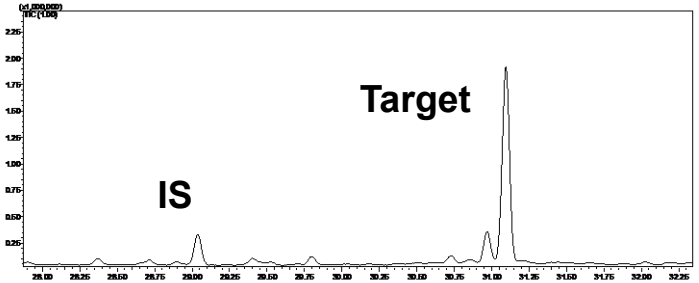
Ideal internal standard:

- It must be well separated from the components in the sample
- It must *not* be present naturally in the sample(s).
- It must have similar chemical properties to the component(s) of interest.
- It must be added in a amount similar to the compounds of interest
- Contribution of noise and interferents should be neglectable

Desirable



Not desirable



Best solution: labelled compounds (usually deuterated)

when **MS** is used

Quantification strategy in instrumental analytical chemistry

- **Internal standard**

: A fixed amount of the internal standard species is added to all samples, standards, and blanks.

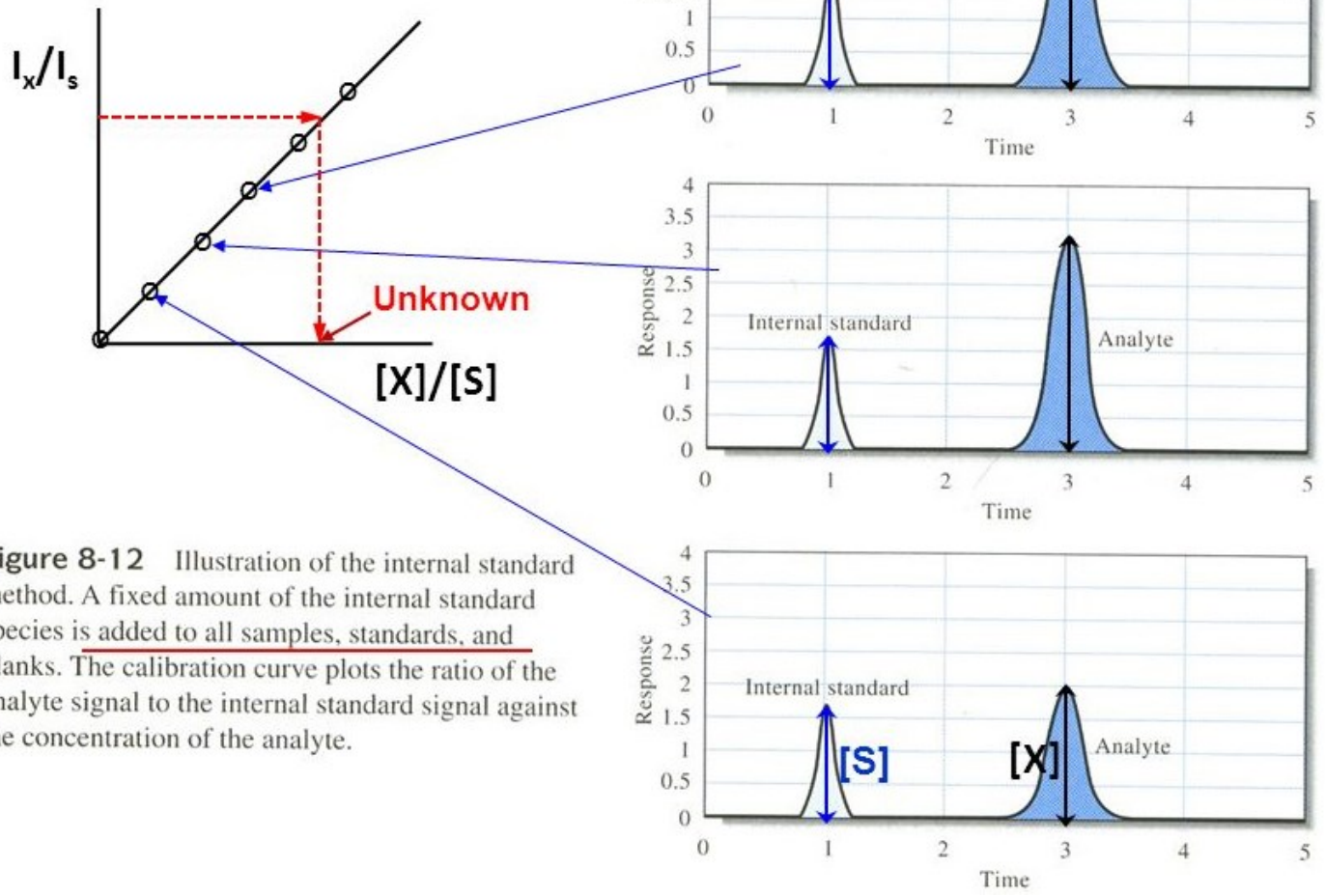
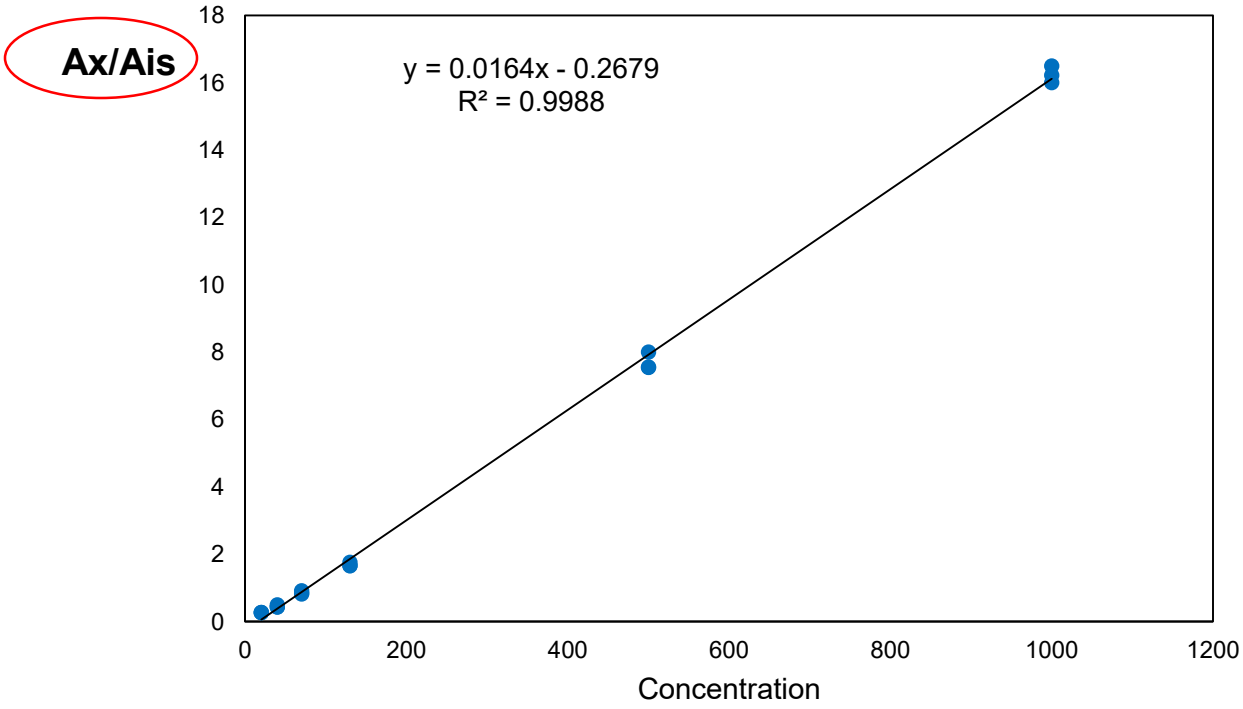


Figure 8-12 Illustration of the internal standard method. A fixed amount of the internal standard species is added to all samples, standards, and blanks. The calibration curve plots the ratio of the analyte signal to the internal standard signal against the concentration of the analyte.

Quantification strategy in instrumental analytical chemistry

- **Internal standard**



Quantification strategy in instrumental analytical chemistry

- **Internal standard**

Advantages

- Quality control at each step of the analytical procedure
- Compensation for **matrix effect**

BUT

Disdvantages

- Sometimes not easy to determine the proper IS

Quantification strategy in instrumental analytical chemistry

- **Matrix-matched calibration**
  - Targeted analytes are added on a matrix where the analytes are absent or present at very low concentration
  - Each concentration level of the calibration curve undergoes the entire analytical procedure

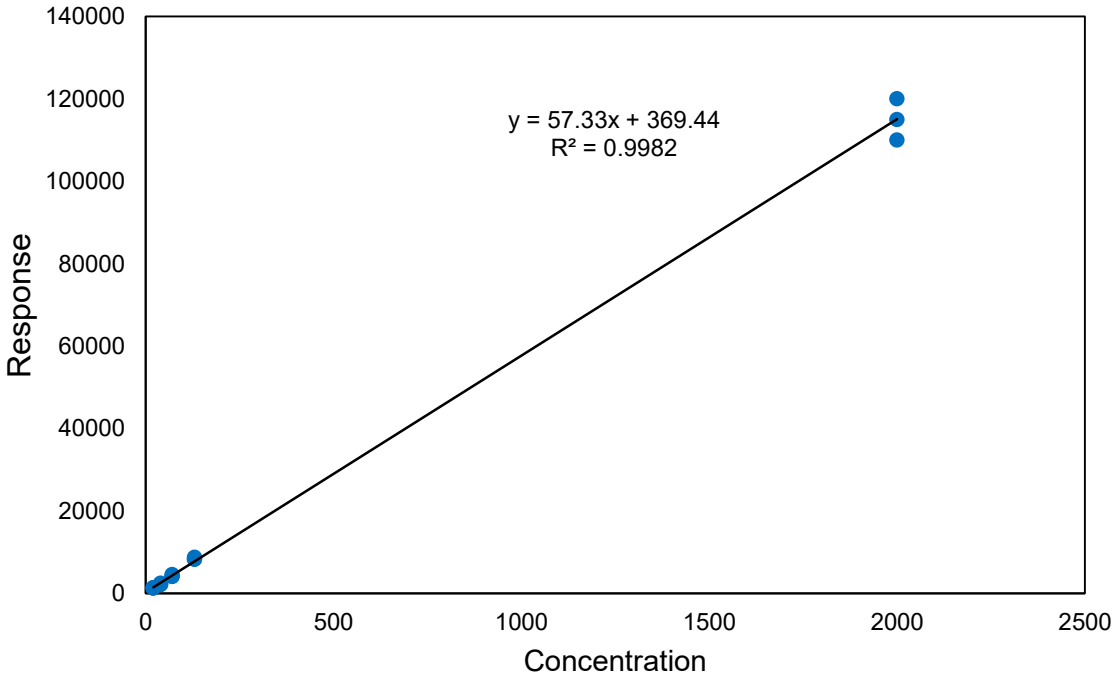
✓ Take into account recovery

✓ Consider matrix effect

Quantification strategy in instrumental analytical chemistry

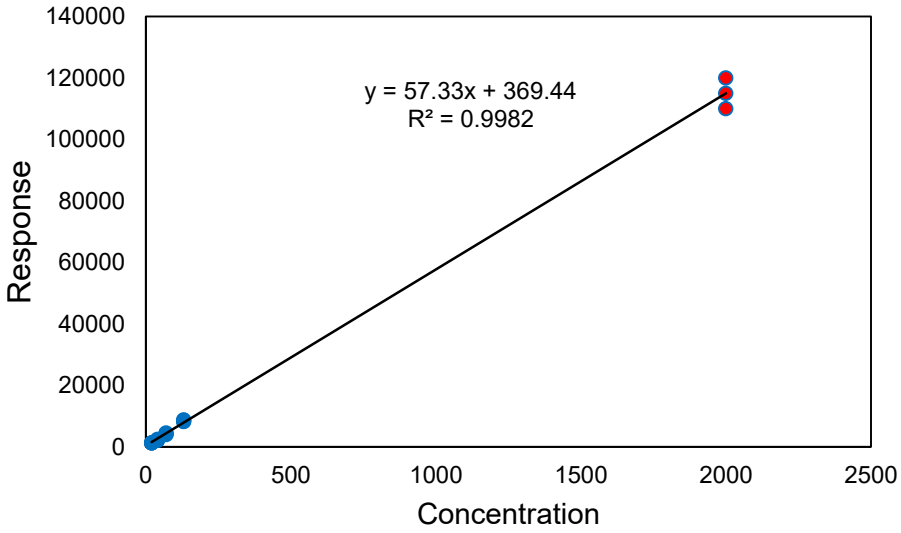
Some important TIPS!

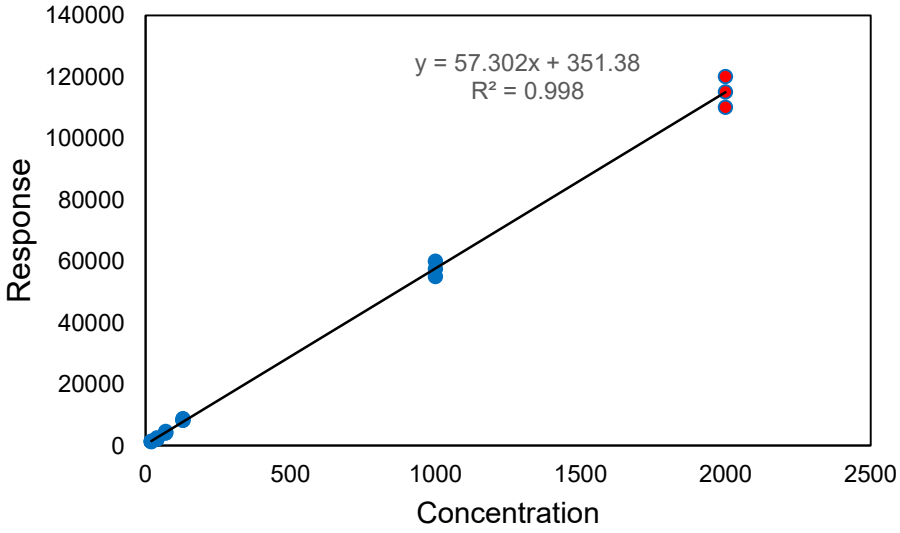
Concentration levels to build the calibration curve should be well distributed within the tested range

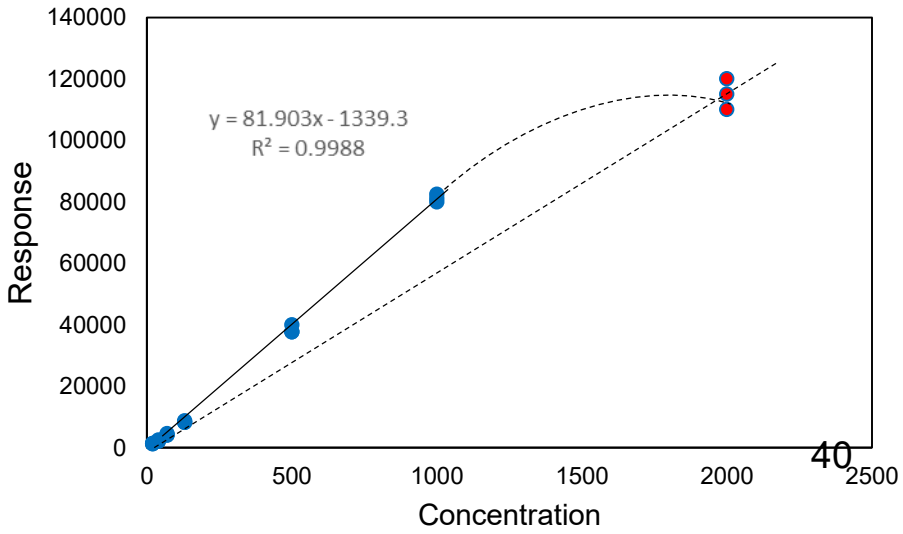


Quantification strategy in instrumental analytical chemistry

Some important TIPS!



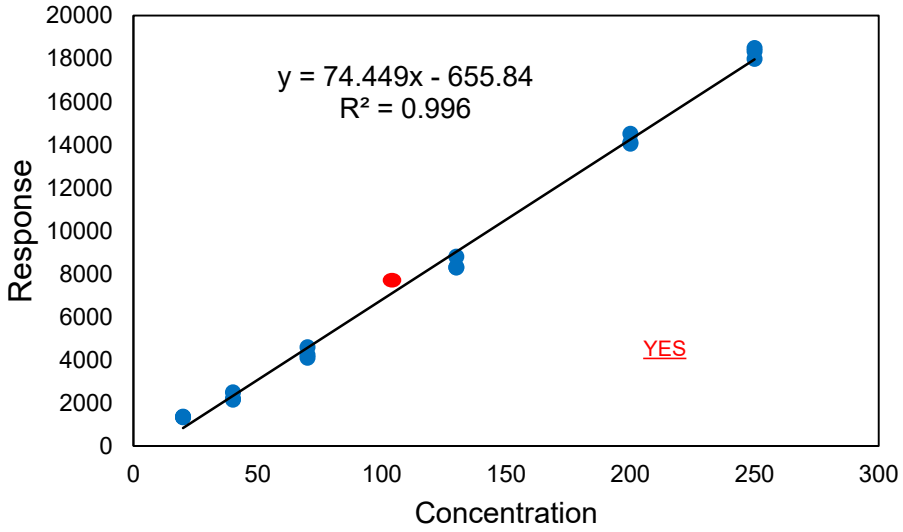




Quantification strategy in instrumental analytical chemistry

Some important TIPS!

Samples to be quantified should be within the linearity range tested



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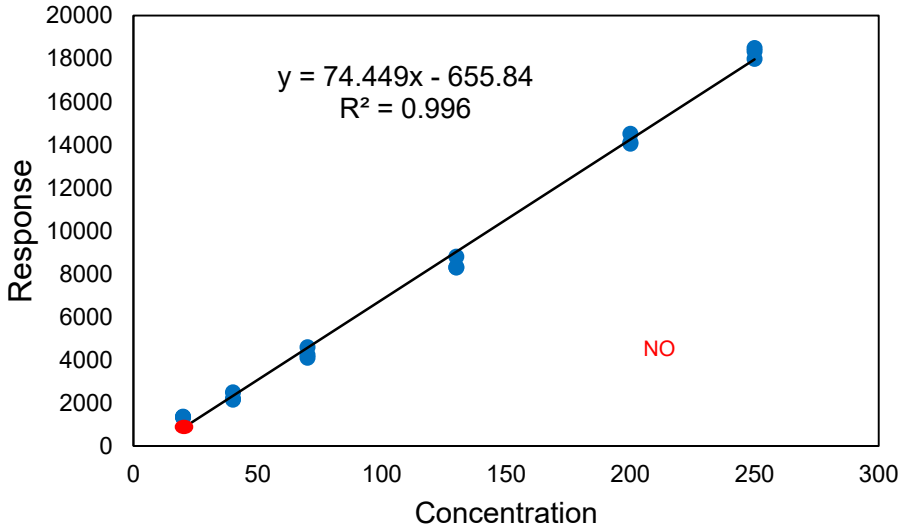

Quantification strategy in instrumental analytical chemistry

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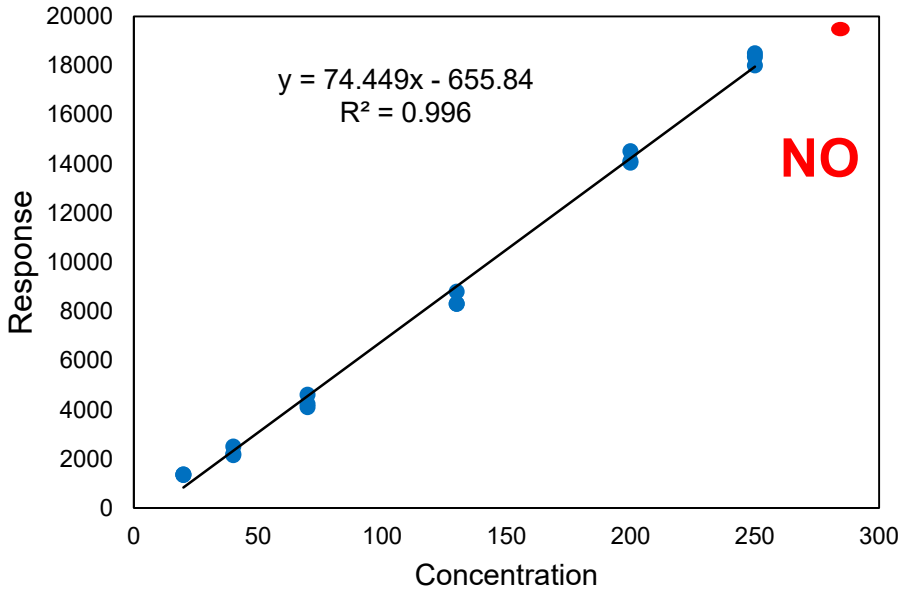
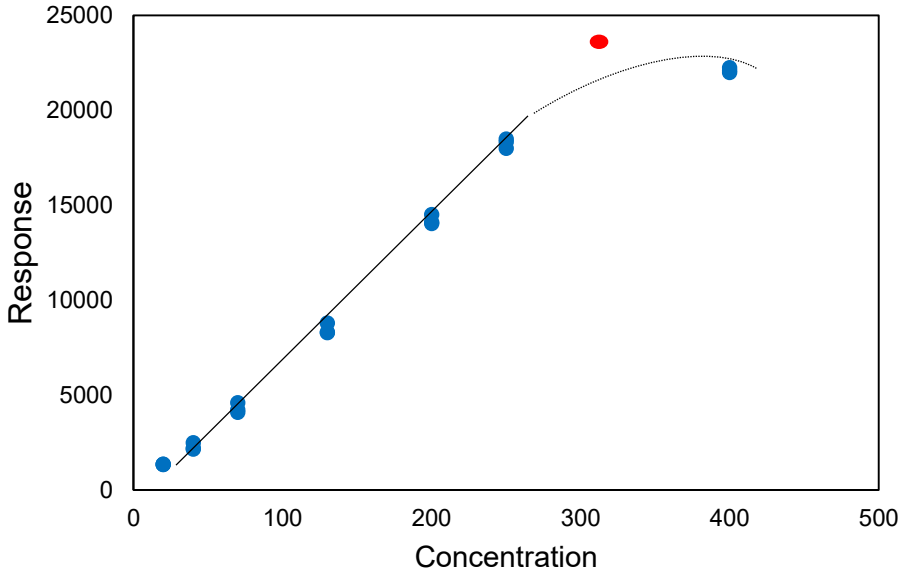
Samples to be quantified should be within the linearity range tested





Quantification strategy in instrumental analytical chemistry

- **Standard addition**

Sample is spiked with known quantity of compound of interest

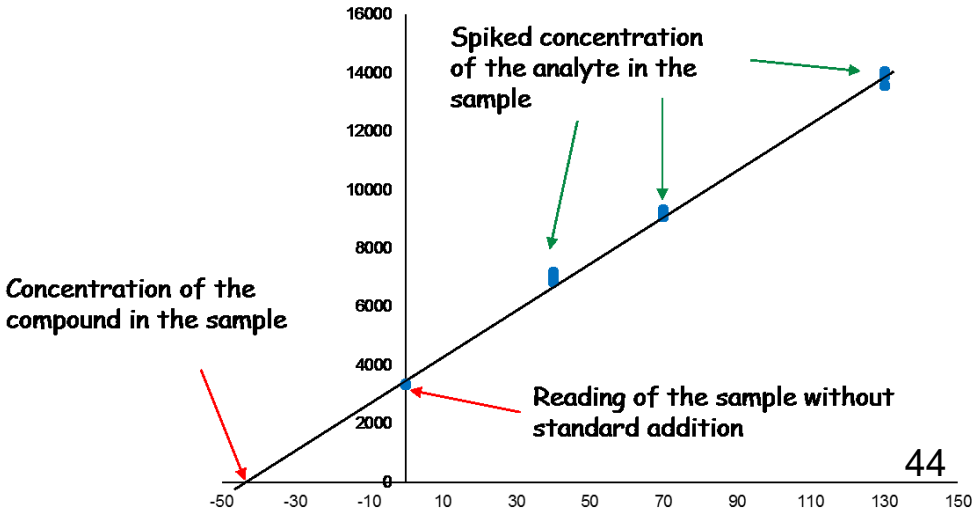
Advantages

- Take into account the matrix effect

BUT

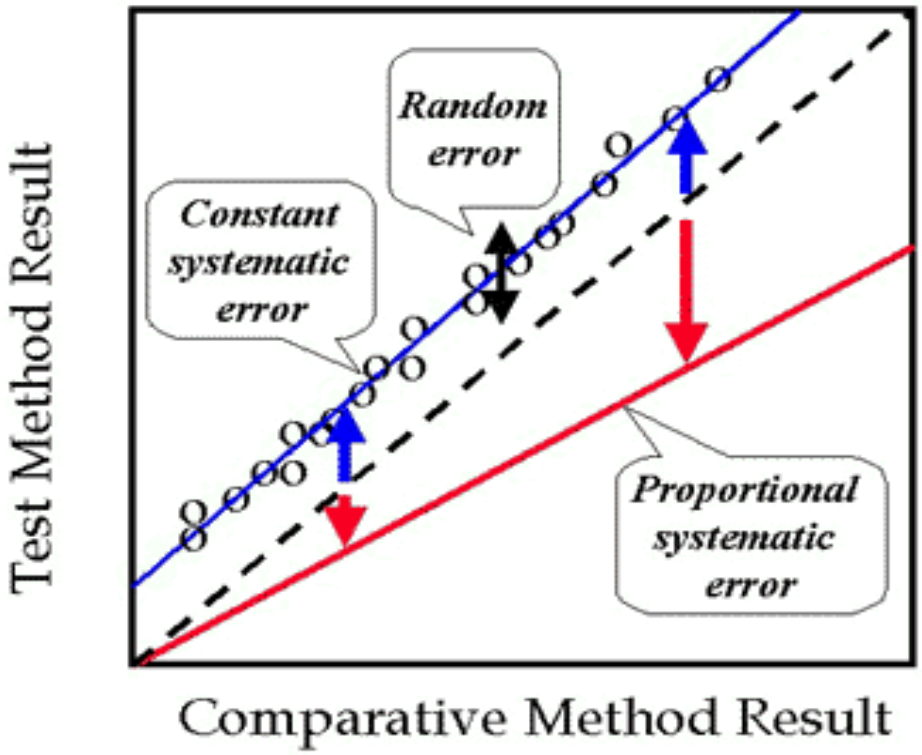
Disdvantages

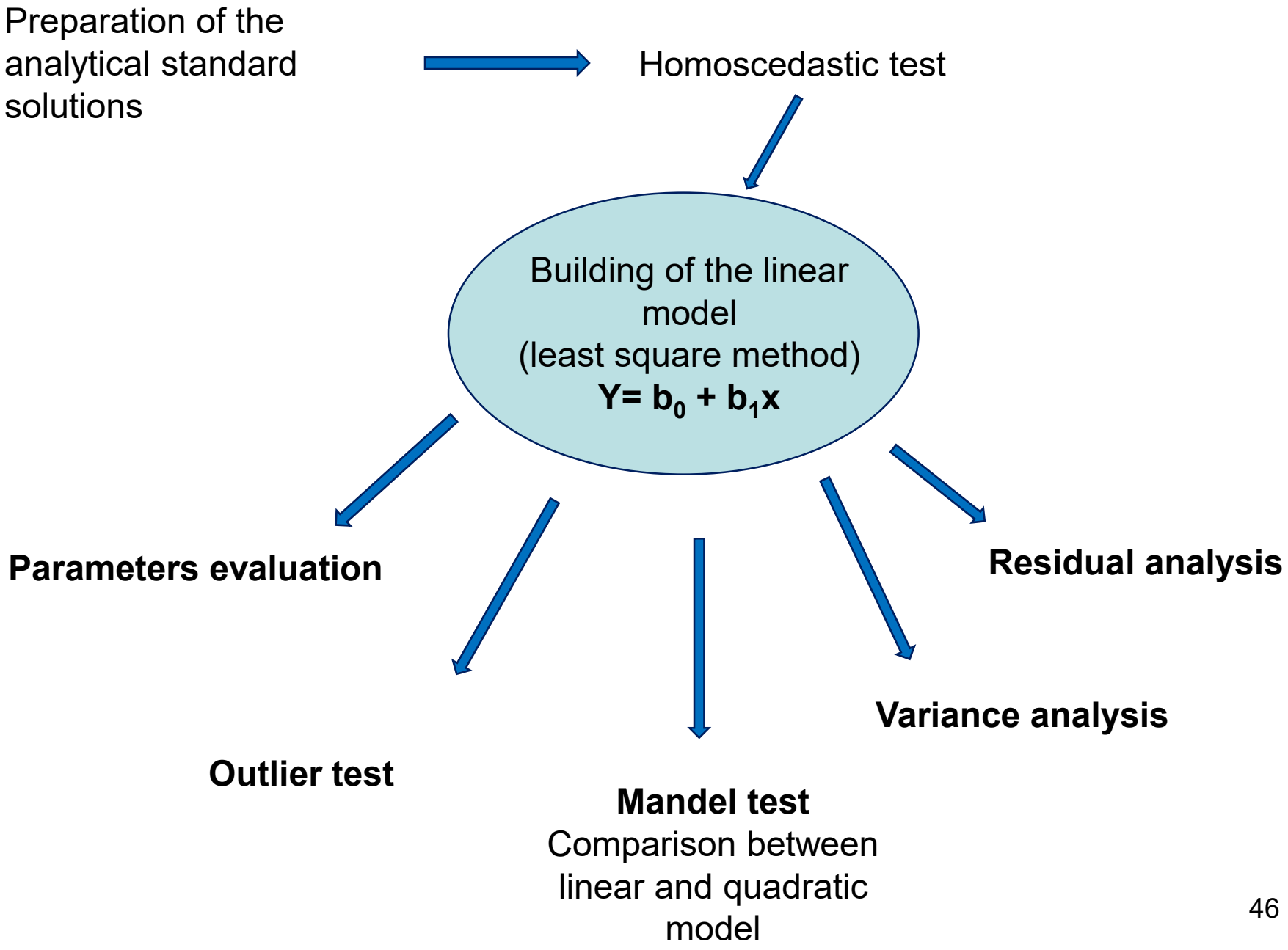
- It is labor intensive
- Separate calibration is required for each sample
- Linear response is required



Quantification strategy in instrumental analytical chemistry

- **Linearity**




Preparation of the analytical standard solutions
Homoscedastic test
Building of the linear model (least square method)
Y= b0 + b1x
Parameters evaluation
Residual analysis
Variance analysis
Outlier test
Mandel test
Comparison between linear and quadratic model

Least square method

- It allows to estimate the coefficient b_0 e b_1 in the linear model

$$Y=b_0 + b_1 X$$

- For each point it calculate the residual value

$$\varepsilon i = Yi \text{ observed} - Yi \text{ calculated} = Yi \text{ observed} - b_0 - b_1 X i$$

- The calculation of the coefficient b0 e b1 is done by minimizing the sum of the sqaure of the residues

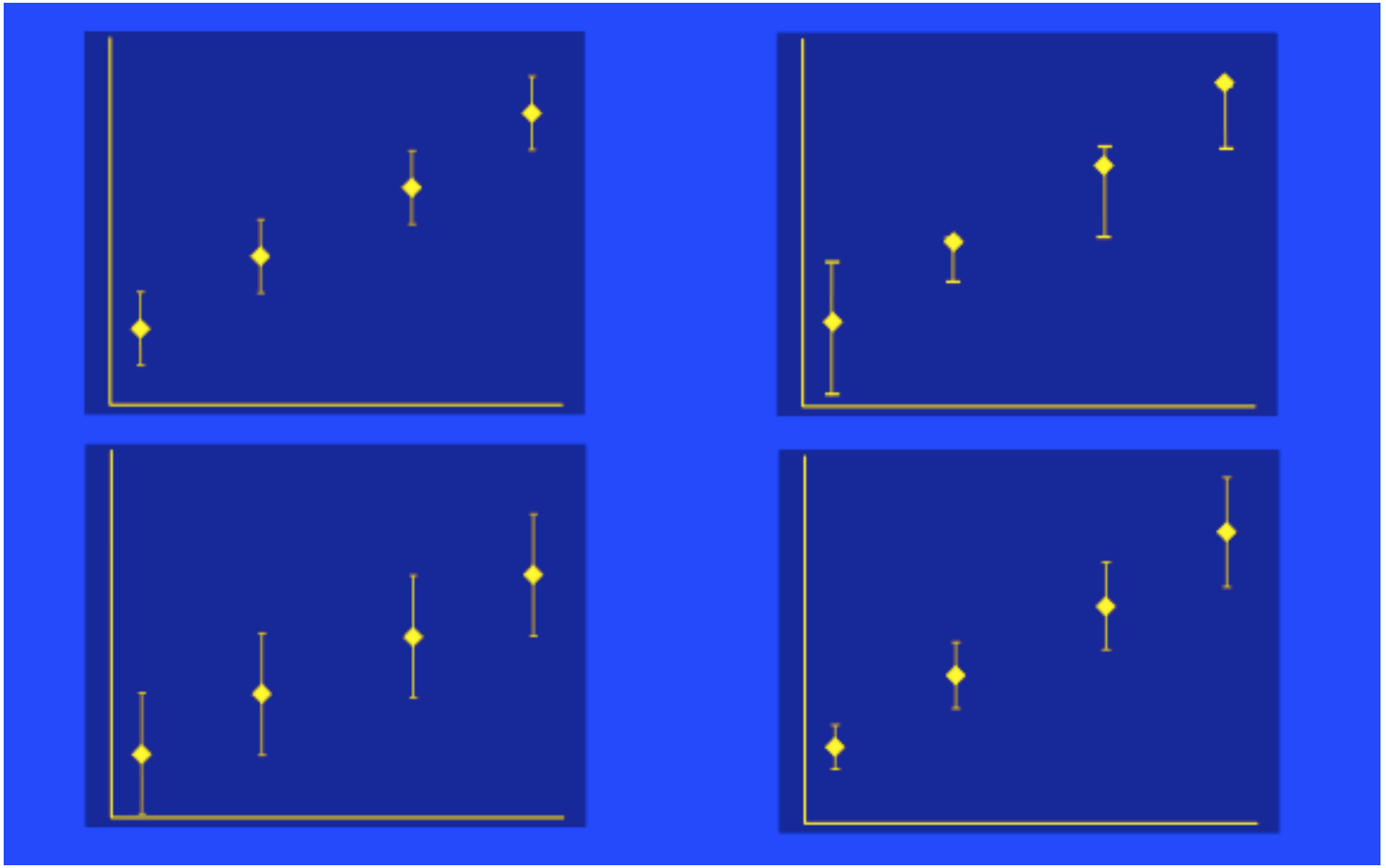
Least square method: limitations

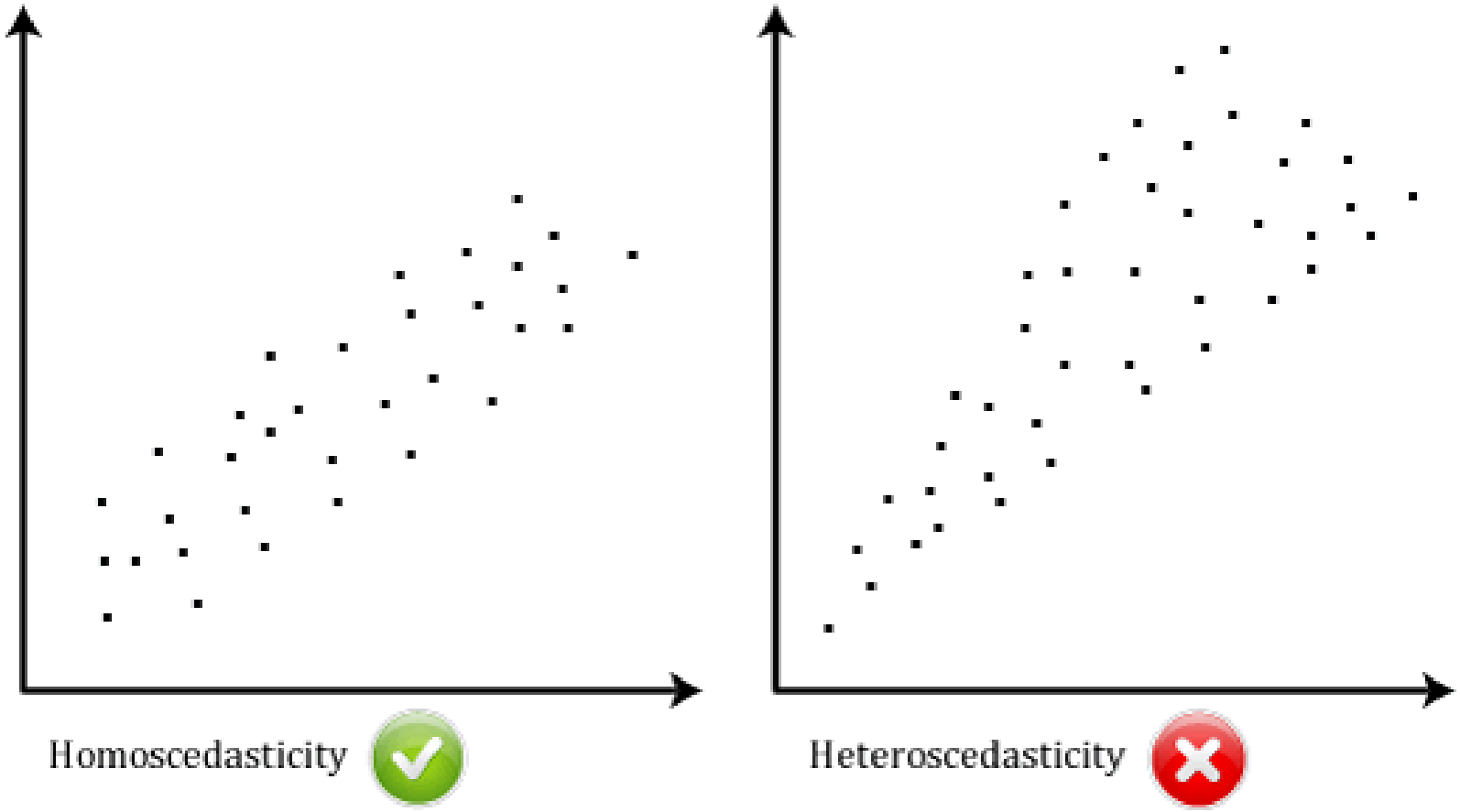
- The error on the x value should be neglectable compared to the error on the y value
- The residuals ε_i have to be indipendent variables with average 0 and variance σ^2 (normal distribution)
- All the residual ε_i have to have the same variance σ^2 (homoscedastic condition)

Homoscedastic of variance

If not the results are not precise and accurate due to the variation of the slope of the line within the tested interval

Homoscedasticity **Heteroscedasticity**

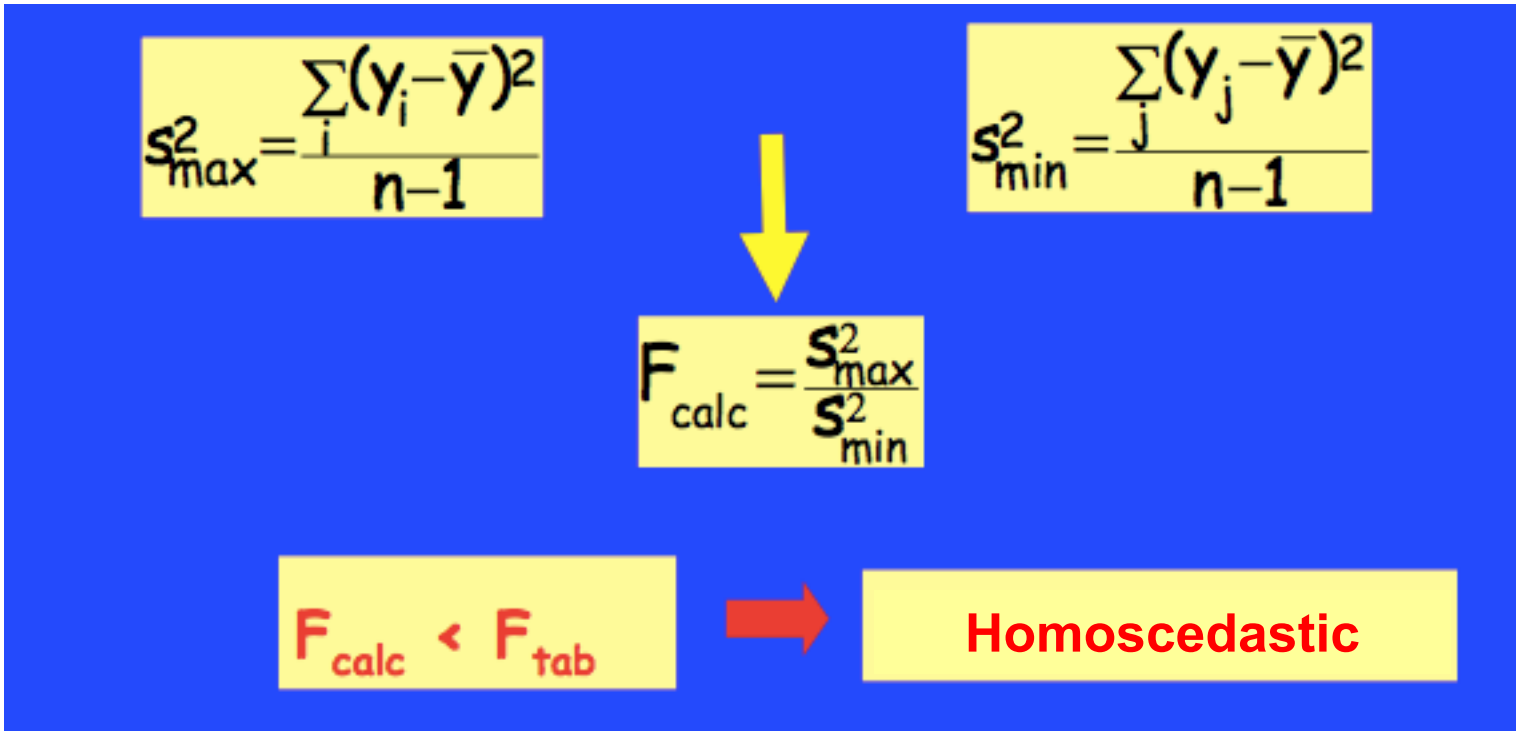



Homoscedasticity
Heteroscedasticity

Homoscedastic test

Comparison between replicates at the extreem of the measure tested (F-test)

Assumption: whether homoscedastic is not meet is assumed to be due to increasing disomogeneity , thus only minumum and maximum are tested



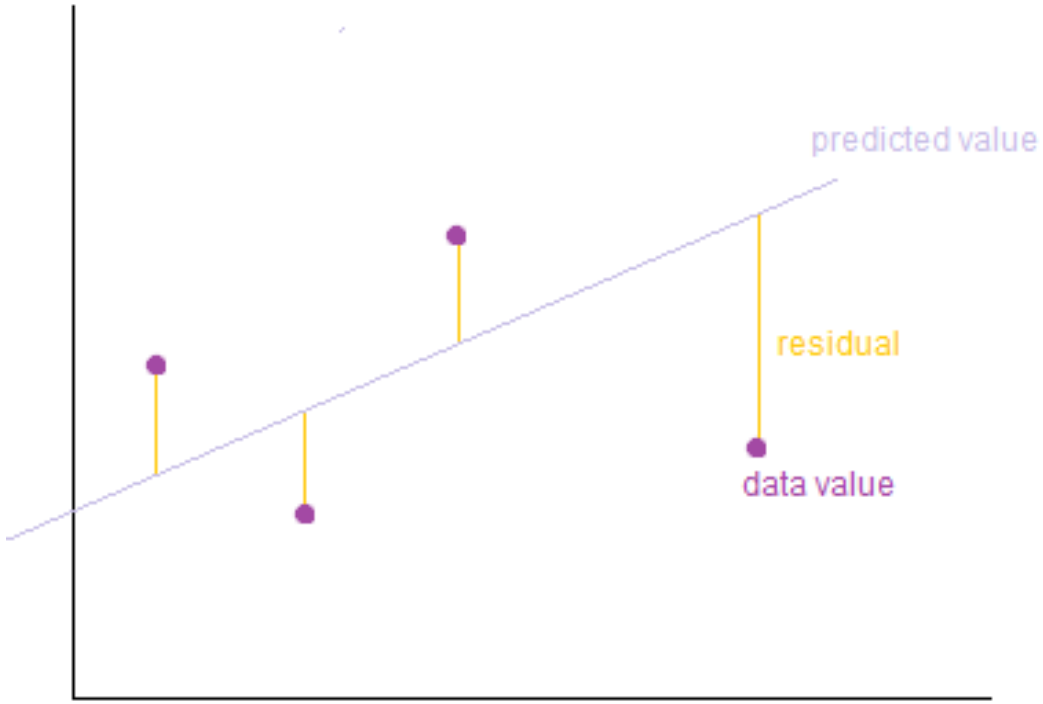
See excel file

Whether not homoscedastic

- Reduce the calibration range
- Used a different model (e.g; weighted squared method)
- Transform the variables (e.g. log transform)

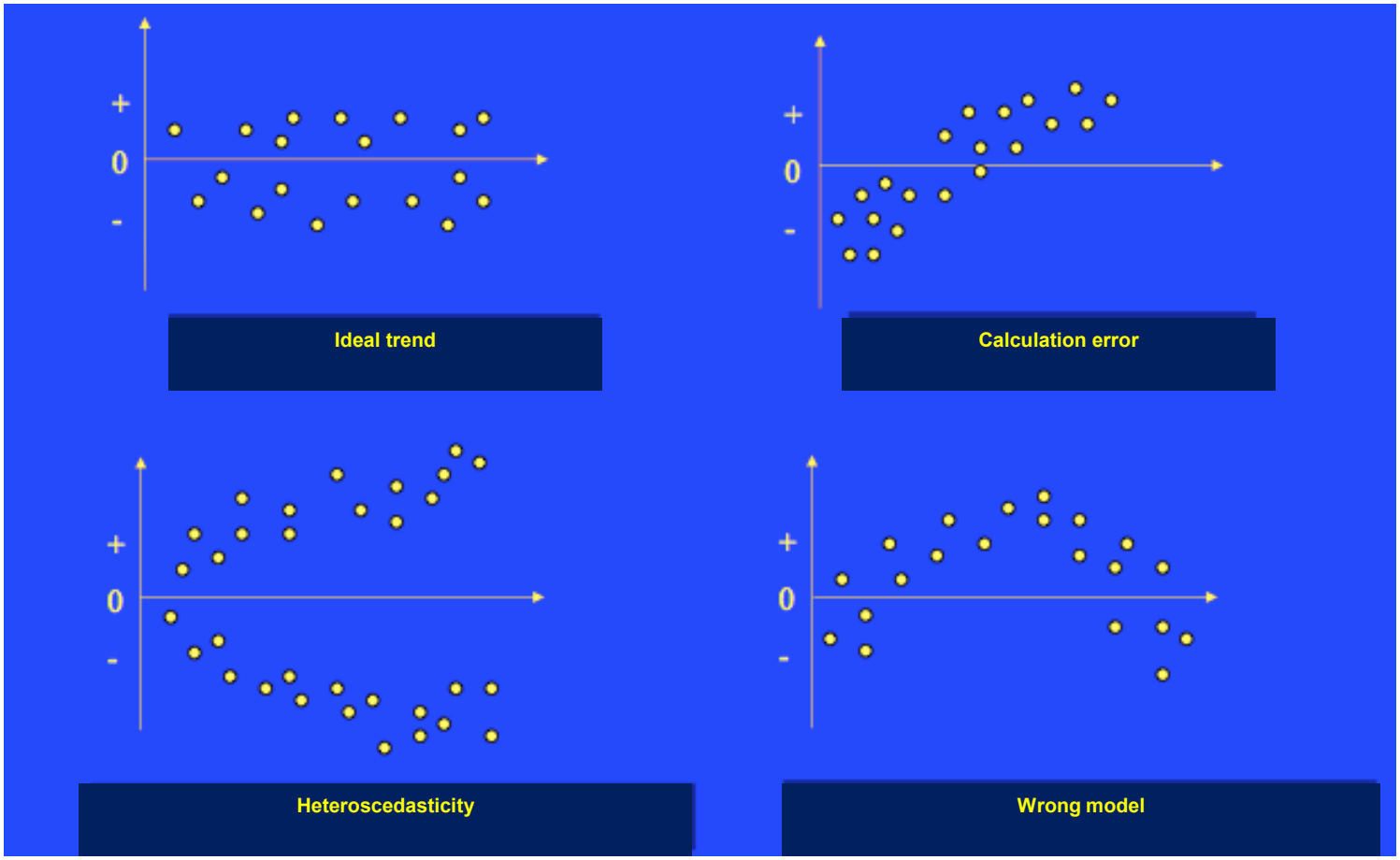
Analysis of the model

- Linearity:
  - Visual analysis of the residual plot

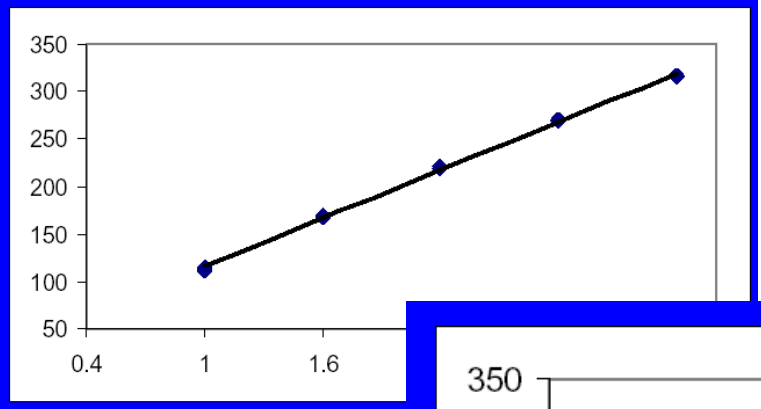


Analysis of the model

- Linearity:
  - Visual analysis of the residual plot

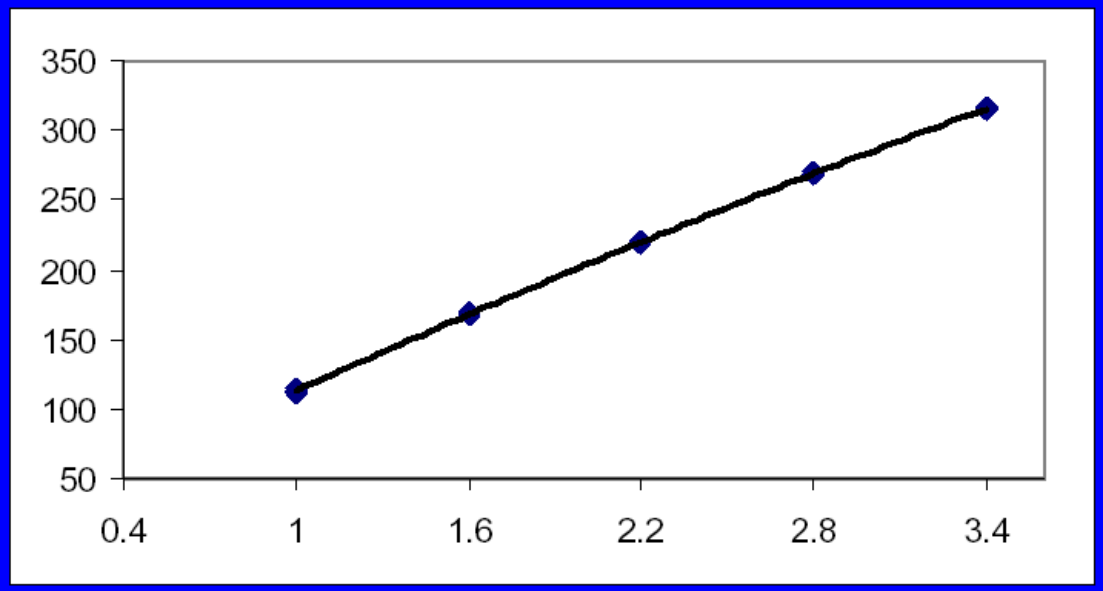


X	Y
1	113
1	112
1	114
1.6	167
1.6	168
1.6	169
2.2	219
2.2	220
2.2	221
2.8	268
2.8	269
2.8	270
3.4	315
3.4	316
3.4	317



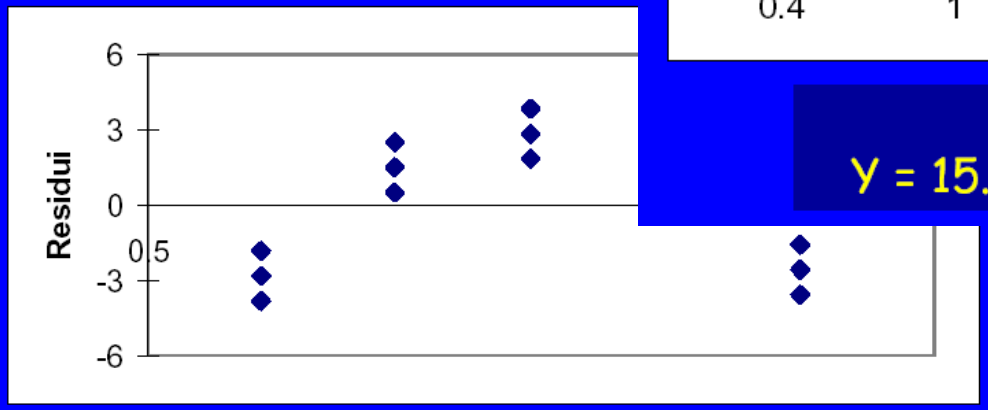
$Y = 31.3 + 84.5\ X$

Based on the R^2 is the model linear?



Quadratic model

$Y = 15.768 + 101.09\ X - 3.7698\ X^2$



Residual analysis

Linearity test
Mandel test

Linear model

$$y = b_0 + b_1 x$$

$$SS_{lin} = \sum (y_{oss(l)} - y_{calc(l)})^2$$

$$MS_{lin} = \frac{SS_{lin}}{n-2}$$

Quadratic model

$$y = b_0 + b_1 x + b_2 x^2$$

$$SS_{quad} = \sum (y_{oss(q)} - y_{calc(q)})^2$$

$$MS_{quad} = \frac{SS_{quad}}{n-3}$$

$$F_{calc} = \frac{SS_{lin} - SS_{quad}}{MS_{quad}}$$

$F_{calc} < F_{tab}(1, n-3)$ → Linear model

$F_{calc} > F_{tab}(1, n-3)$ → Quadratic model

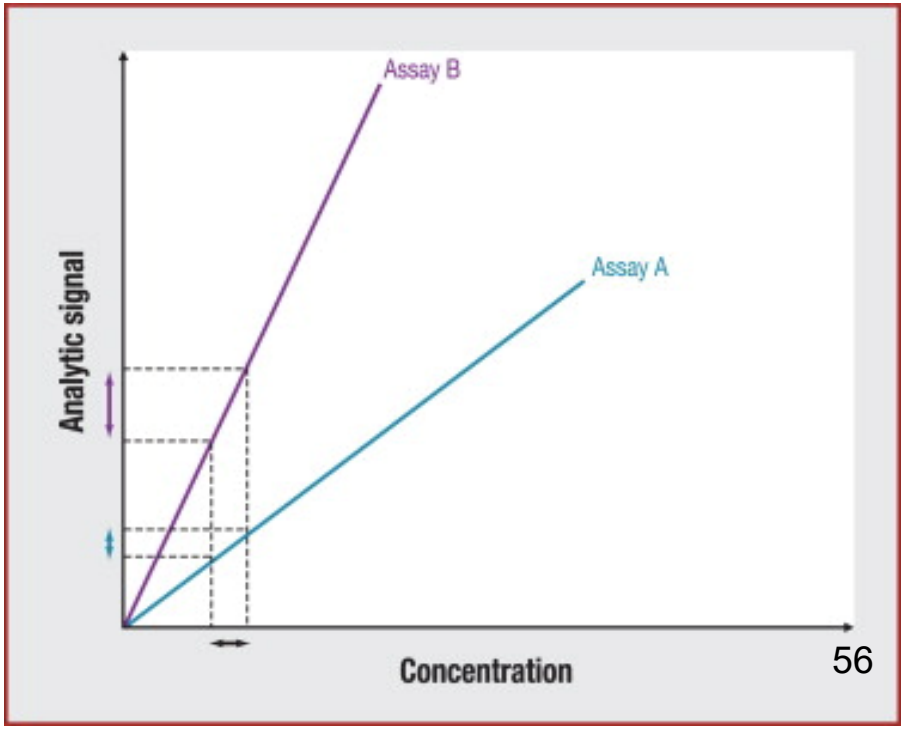
Quantitative Analytical Method

LOD&LOQ....& Sensitivity:

Sensitivity:

The change in the response of the measuring instrument divided by the corresponding change in concentration

➡ Slope of the calibration curve



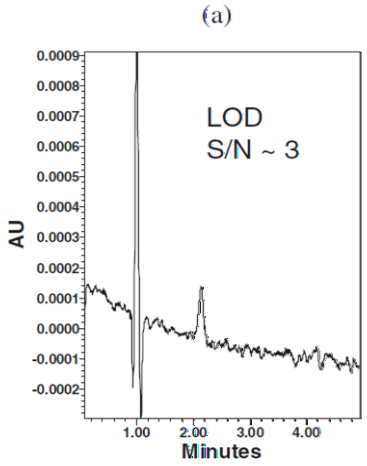
Quantitative Analytical Method

LOD&LOQ....& Sensitivity:

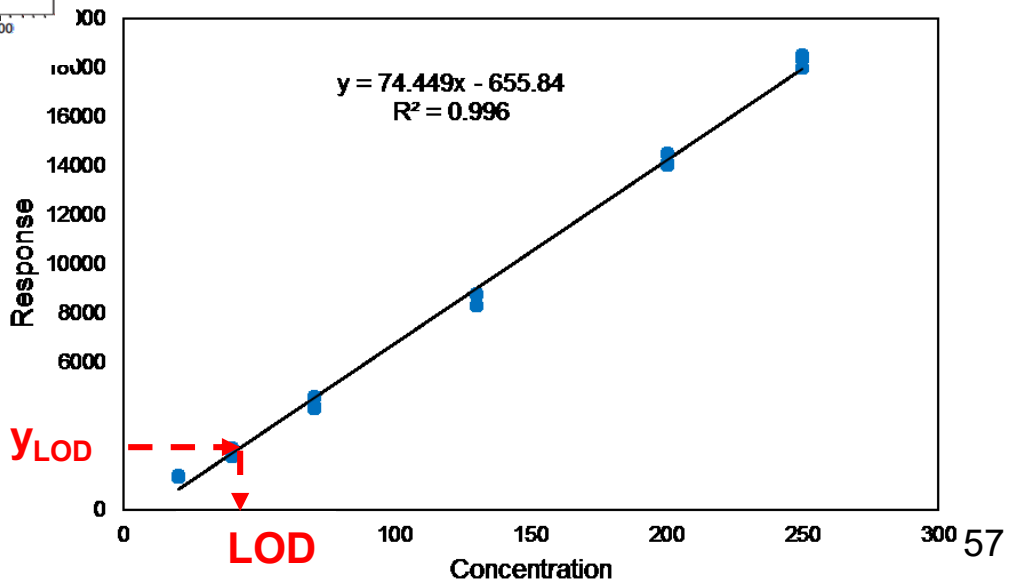
LOD (Limit of Detection):

is the smallest amount or concentration of analyte that can be detected.

- 3 × S/N



- $y_{LOD} = y_{blank} + 3\,\sigma_{blank}$



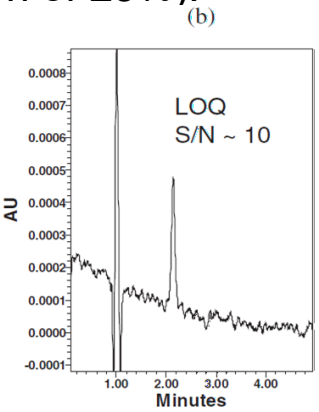
Quantitative Analytical Method

LOD&LOQ....& Sensitivity:

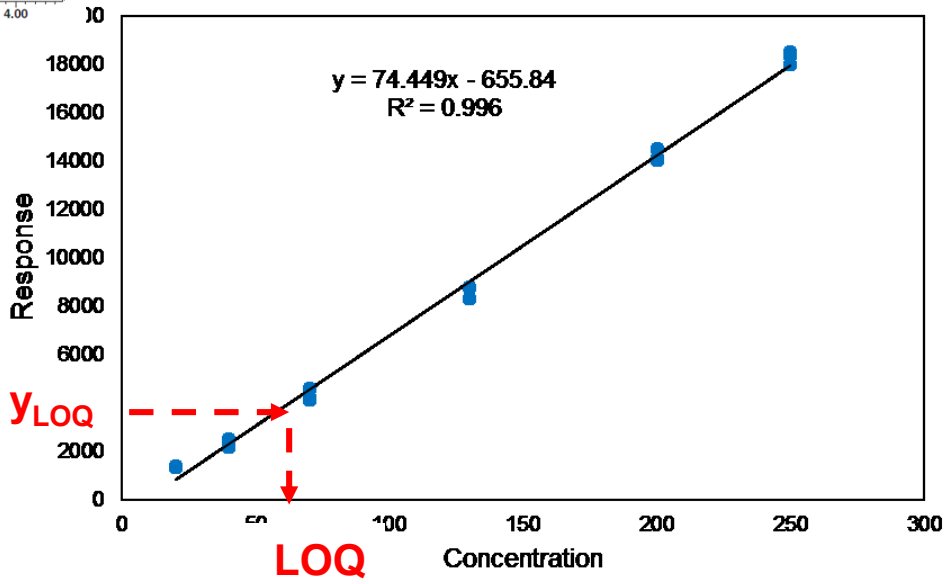
LOQ (Limit of Quantification):

is the lowest level that an analyte can be quantitated with some degree of certainty (e.g., with a precision of ±5%).

- 10 × S/N



- $y_{LOQ} = y_{blank} + 10\ \sigma_{bla}$



Quantitative Analytical Method

Decision Limit (CCα) & Detection capability (CCß):

These terms are applicable for the measurement of organic residues, contaminants and chemical elements in live animals and animal products, as regulated within the EU by the Council Directives 96/23/EC, 2002/657/EC and 2003/181/EC.

The Commission distinguishes:

- `**Group A** substances', for which **NO** permitted limit (PL) (maximum residue level, **MRL**) has been established, and
- `**Group B** substances' having a fixed PL.

NOTE: these terms apply specifically to inspection of animals and fresh meat for the presence of residues of veterinary drugs and specific contaminants and are therefore different from LOD and LOQ

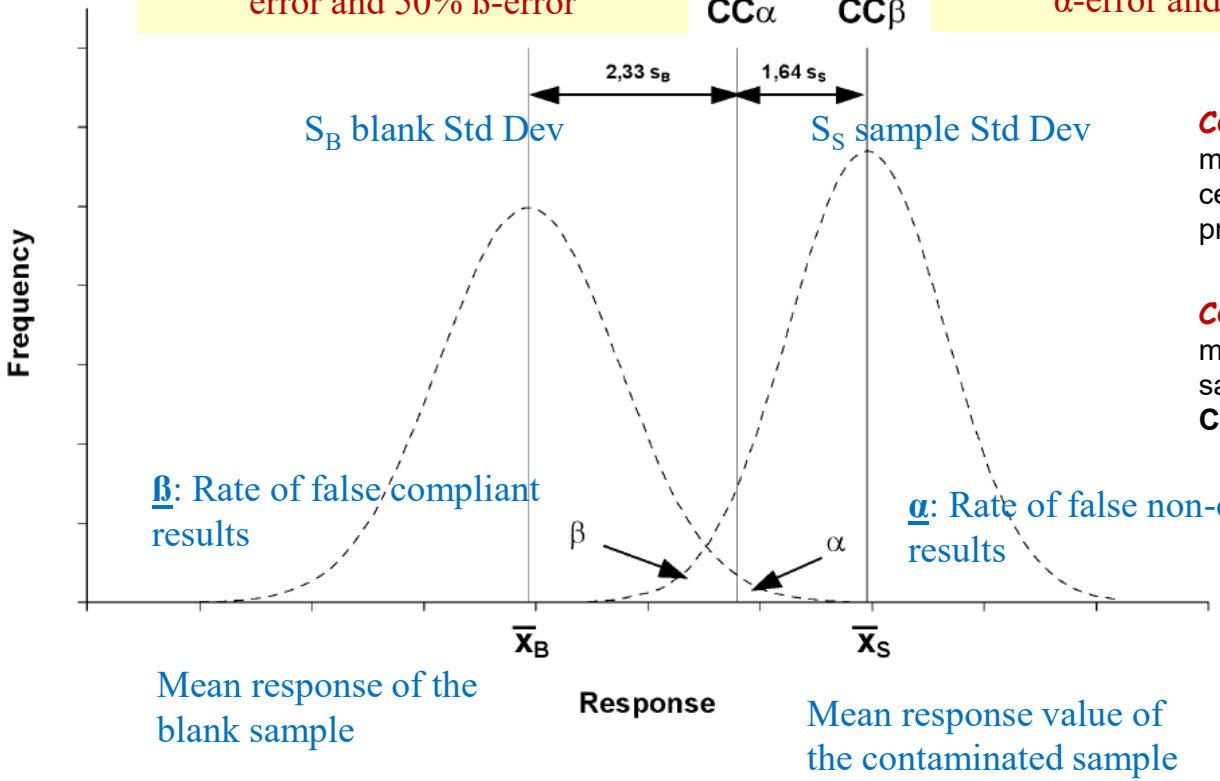
Quantitative Analytical Method

Decision Limit (CCα) & Detection capability (CCß):

- `**Group A** substances', for which **NO** permitted limit (PL) (maximum residue level,

CCα Response with a given α-error and 50% ß-error

CCß Response with a very small α-error and a ß-error



CCα is the lowest concentration level at which the method can discriminate with a statistical certainty of **1-*α*** that the particular analyte is present ($CC_\alpha = x_{bl} + 2.33\ s_{sample}$).

CCß is the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of ***β*** ($CC_\beta = CC_\alpha + 1.65\ s_{sample}$).

CCα and **CCβ** are comparable with **LOD** and **LOQ**, respectively, as their concentrations correspond to measured signals laying y times above the blank signal.

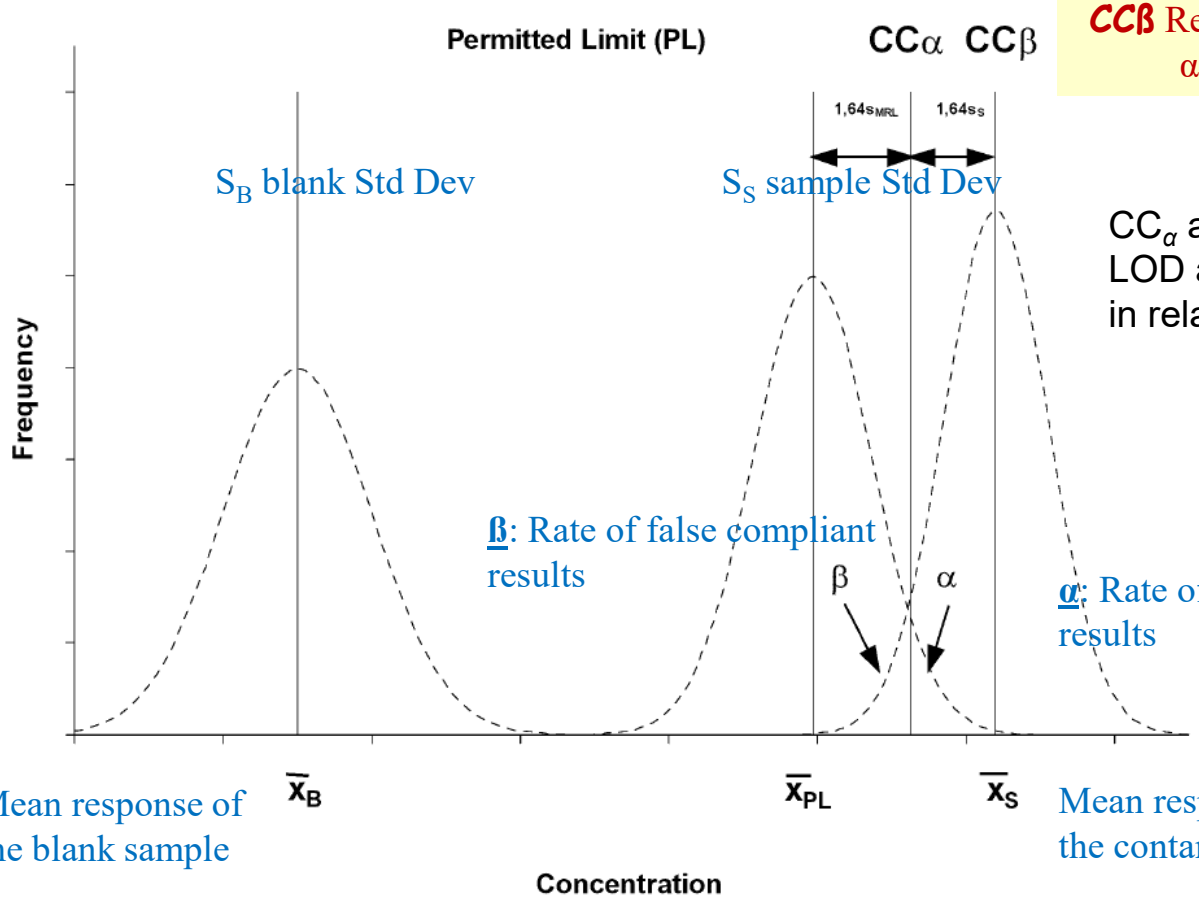
Quantitative Analytical Method

Decision Limit (CCα) & Detection capability (CCß):

- `**Group B** substances' having a fixed PL (Permitted Limit).

CCα Response with a given α-error and 50% ß-error

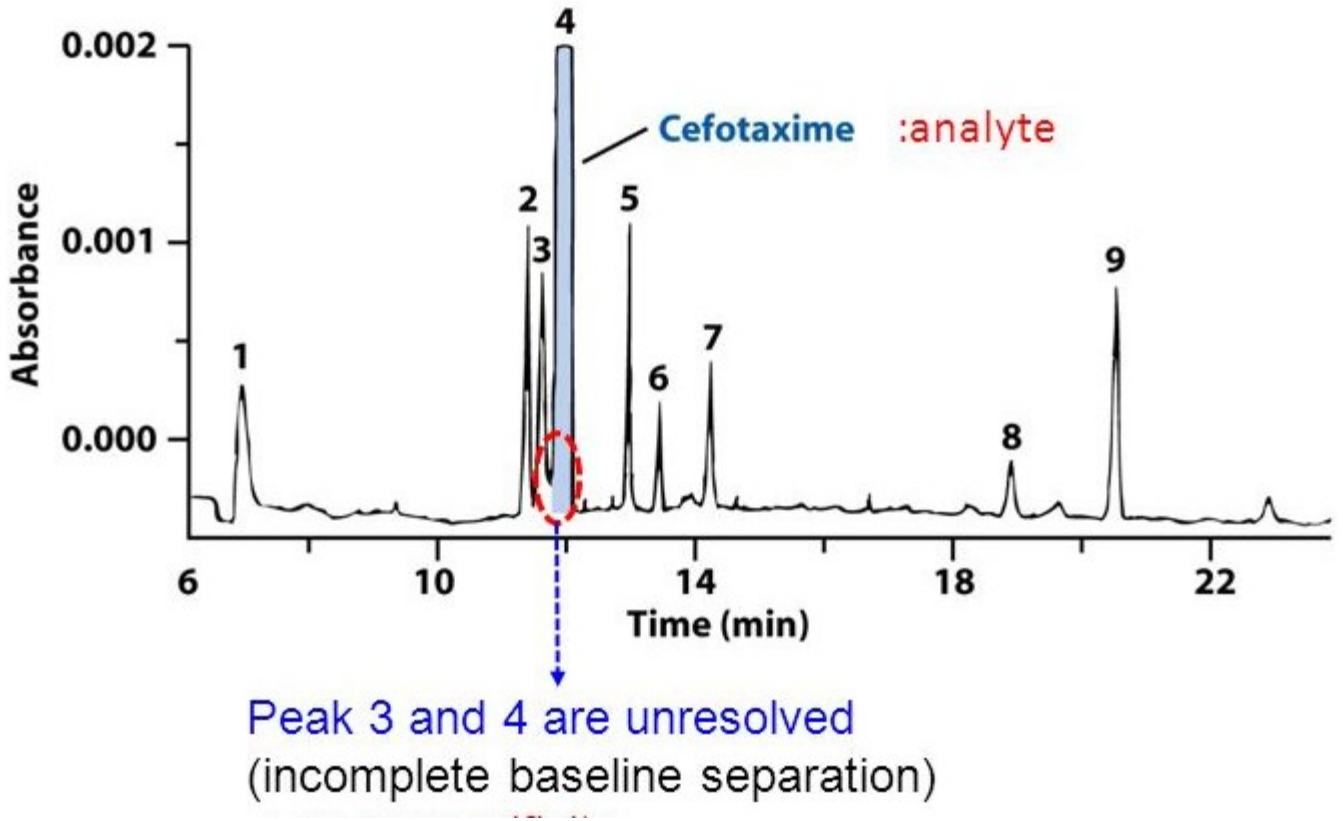
CCß Response with a very small α-error and a ß-error



CC_α and CC_β are not related to LOD and LOQ but are expressed in relation to this PL.

Quantitative Analytical Method

Specificity: ability of the method to distinguish the analytes from everything else



Quantitative Analytical Method

Specificity: ability of the method to distinguish the analytes from everything else

Needs to be proved, cannot be expressed in anyway:

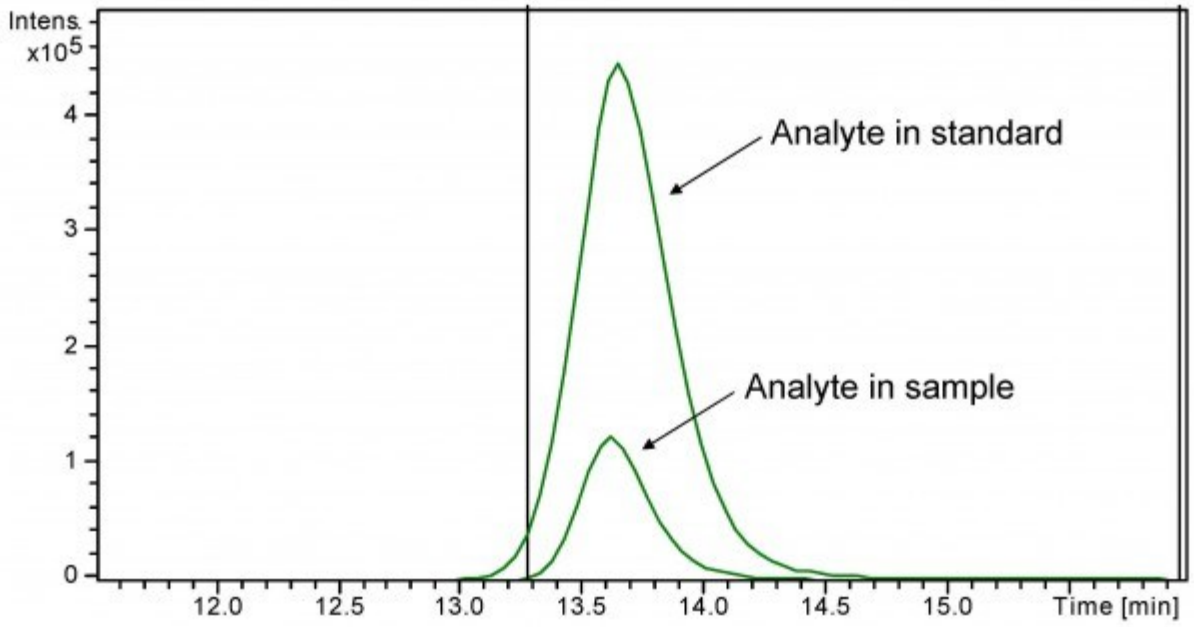
Identification tests: % of correct classification

Quantitative tests: % of recovery

Quantitative Analytical Method

- **Matrix effect:** is defined as the combined effect of all components of the samples other then analyte

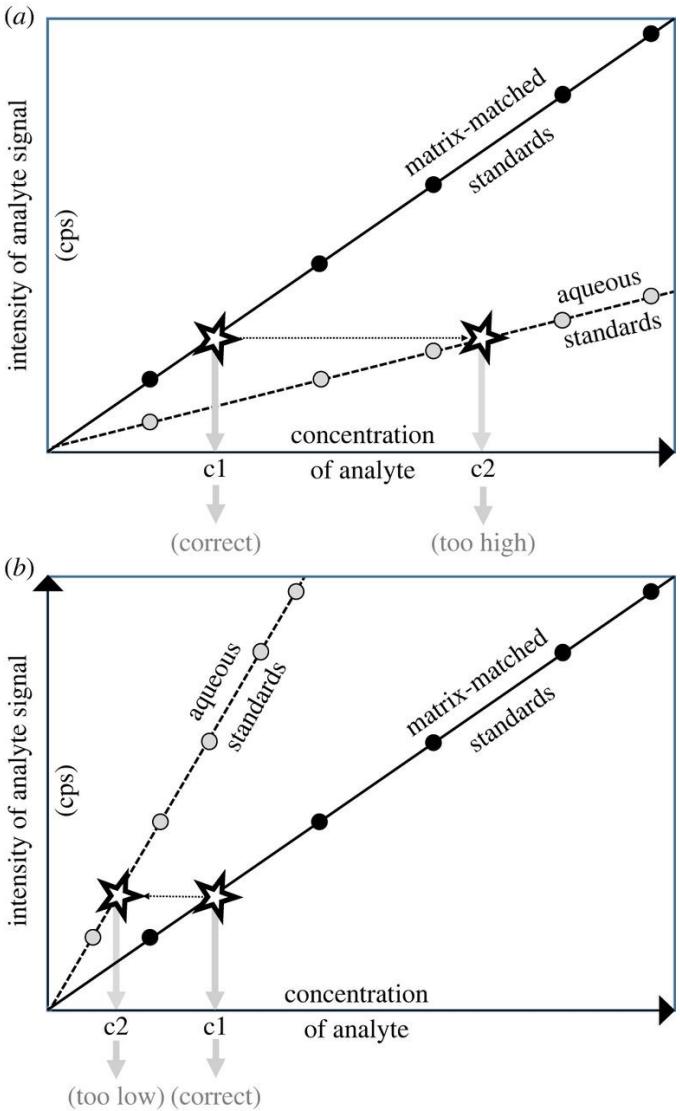
How does matrix effect look like?



Matrix effect: Concentrations are the same, but peak areas are vastly different!

Quantitative Analytical Method

- **Matrix effect:** is defined as the combined effect of all components of the samples other then analyte



$$\mathrm{ME}(\%) = 100 - \frac{B}{A} \times 100$$

A represents the average peak area of the standard solution (n=5)

B represents the average peak area of a sample extract at the same concentration of the standard (n=5).

A and **B** can be the slope of the two calibration curves

Quantitative Analytical Method

- **Robustness/Ruggedness:** is "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Ruggedness provides an indication of the method's reliability during normal usage"

Quick Reference 8 – Ruggedness

What to do	How many times	What to calculate/determine from the data	Comments
Identify variables which could have a significant effect on method performance. Set up experiments (analysing RMs or test samples) to monitor the effect on measurement results of systematically changing the variables.	Most effectively evaluated using experimental designs. E.g. 7 parameters can be studied in 8 experiments using a Plackett-Burman experimental design [74].	Determine the effect of each change of condition on the measurement results. Rank the variables in order of the greatest effect on method performance. Carry out significance tests to determine whether observed effects are statistically significant.	Design quality control or modify the method in order to control the critical variables, e.g. by stating suitable tolerance limits in the standard operating procedure.

General Method Performance Requirments

COMMISSION

COMMISSION DECISION

of 12 August 2002

implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

(notified under document number C(2002) 3044)

(Text with EEA relevance)

(2002/657/EC)

Article 1

Subject matter and scope

This Decision provides rules for the analytical methods to be used in the testing of official samples taken pursuant to Article 15(1), second sentence, of Directive 96/23/EC and specifies common criteria for the interpretation of analytical results of official control laboratories for such samples.

This Decision shall not apply to substances for which more specific rules have been laid down in other Community legislation.

General Method Performance Requirments

SAMPLING AND ANALYSIS	
For *dioxins*	Regulation 1883/2006
For *nitrates*	Regulation 1882/2006
For *metals* **and** *3-CPD*:	Regulation 333/2007
For *inorganic tin*	
For *PAHs*	
For *aflatoxins*	Regulation 401/2006
For *ochratoxin A*	
For *patulin*	
For *fusarium toxins*	

General Method Performance Requirments

Trueness

Minimum trueness of quantitative methods

Mass fraction	Range
≤ 1 µg/kg	– 50 % to + 20 %
> 1 µg/kg to 10 µg/kg	– 30 % to + 10 %
≥ 10 µg/kg	– 20 % to + 10 %

When no such CRMs are available, it is acceptable that trueness of measurements is assessed through recovery of additions of known amounts of the analyte(s) to a blank matrix. Data corrected with the mean recovery are only acceptable when they fall within the ranges shown in Table 2.

General Method Performance Requirments

Trueness

Minimum trueness of quantitative methods

Mass fraction	Range
≤ 1 µg/kg	– 50 % to + 20 %
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When no such CRMs are available, it is acceptable that trueness of measurements is assessed through recovery of additions of known amounts of the analyte(s) to a blank matrix. Data corrected with the mean recovery are only acceptable when they fall within the ranges shown in Table 2.

Precision

Table 3

Examples for reproducibility CVs for quantitative methods at a range of analyte mass fractions

Mass fraction	Reproducibility CV(%)
1 µg/kg	(*)
10 µg/kg	(*)
100 µg/kg	23
1 000 µg/kg (1 mg/kg)	16

(*) For mass fractions lower than 100 µg/kg the application of the Horwitz Equation gives unacceptable high values. Therefore, the CVs for concentrations lower than 100 µg/kg shall be as low as possible.

General Method Performance Requirments

2.3.3.1. *Chromatographic separation*

For GC-MS procedures, the gas chromatographic separation shall be carried out using capillary columns. For LC-MS procedures, the chromatographic separation shall be carried out using suitable LC columns. In any case, the minimum acceptable retention time for the analyte under examination is twice the retention time corresponding to the void volume of the column. The retention time (or relative retention time) of the analyte in the test portion shall match that of the calibration standard within a specified retention time window. The retention time window shall be commensurate with the resolving power of the chromatographic system. The ratio of the chromatographic retention time of the analyte to that of the internal standard, i.e. the relative retention time of the analyte, shall correspond to that of the calibration solution at a tolerance of ± 0.5 % for GC and ± 2.5 % for LC.

General Method Performance Requirments

Full scan: When full scan spectra are recorded in single mass spectrometry, a minimum of four ions shall be present with a relative intensity of ≥ 10 % of the base peak. The molecular ion shall be included if it is present in the reference spectrum with a relative intensity of ≥ 10 %. At least four ions shall lie within the maximum permitted tolerances for relative ion intensities (Table 5). Computer-aided library searching may be used. In this case, the comparison of mass spectral data in the test samples to that of the calibration solution has to exceed a critical match factor. This factor shall be determined during the validation process for every analyte on the basis of spectra for which the criteria described below are fulfilled. Variability in the spectra caused by the sample matrix and the detector performance shall be checked.

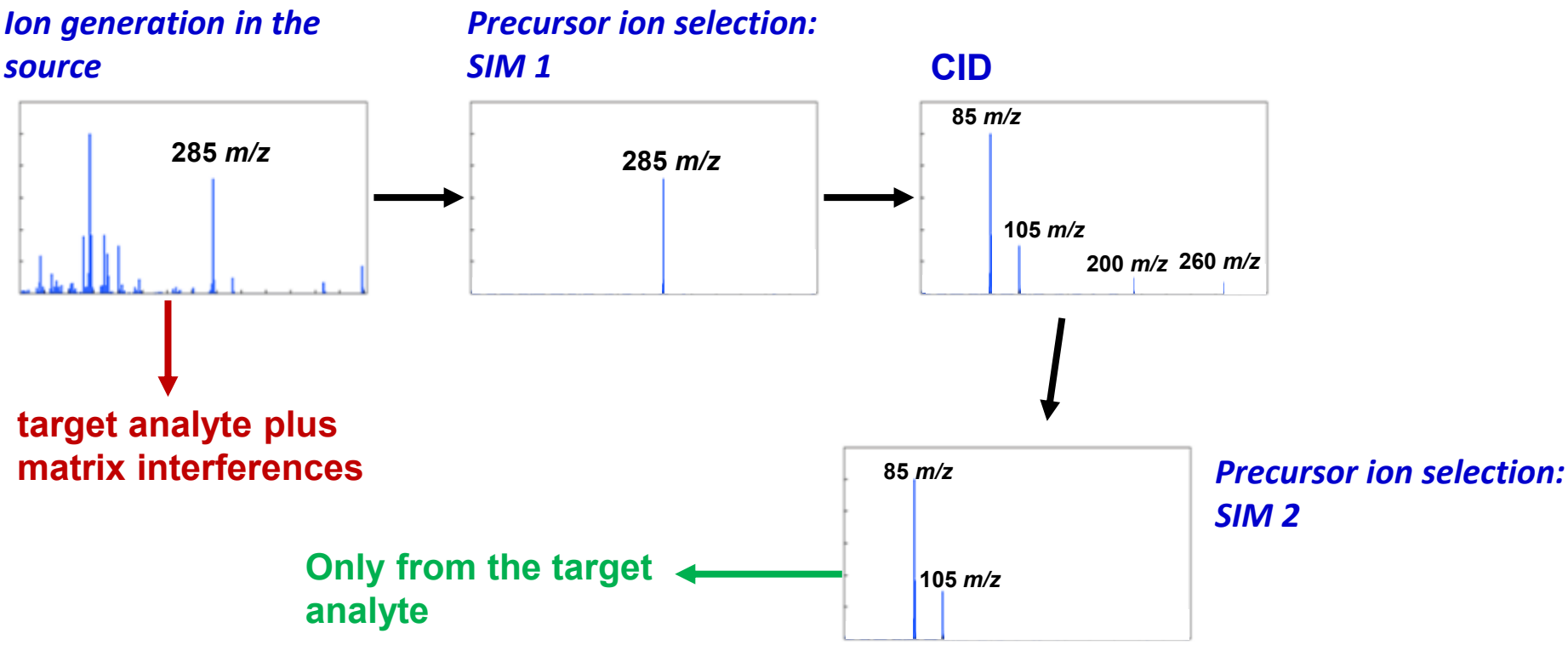
Examples of the number of identification points earned for a range of techniques and combinations thereof (n = an integer)

Minimum number of point necessary 3 or 4 according to the compound *

Technique(s)	Number of ions	Identification points
GC-MS (EI or CI)	N	n
GC-MS (EI and CI)	2 (EI) + 2 (CI)	4
GC-MS (EI or CI) 2 derivatives	2 (Derivative A) + 2 (Derivative B)	4
LC-MS	N	n
GC-MS-MS	1 precursor and 2 daughters	4
LC-MS-MS	1 precursor and 2 daughters	4
GC-MS-MS	2 precursor ions, each with 1 daughter	5
LC-MS-MS	2 precursor ions, each with 1 daughter	5
LC-MS-MS-MS	1 precursor, 1 daughter and 2 granddaughters	5,5
HRMS	N	2 n
GC-MS and LC-MS	2 + 2	4
GC-MS and HRMS	2 + 1	4

* Directive 96/23/EC Annex I: Group A a minimum of 4; Group B a minimum of 3 identification points shall be required.

GC-QqQMS: pre-targeted analysis (MRM)



The QqQ MRM mode enables, very often, the elimination of matrix and chemical background interferences

Modern instrumentation can perform MRM/scan analysis in a simultaneous and rapid manner!

* Directive 96/23/EC Annex I:

ANNEX I

GROUP A — **Substances having anabolic effect and unauthorized substances**

(1) Stilbenes, stilbene derivatives, and their salts and esters

(2) Antithyroid agents

(3) Steroids

(4) Resorcylic acid lactones including zeranol

(5) Beta-agonists

(6) Compounds included in Annex IV to Council Regulation (EEC) No 2377/90 of 26 June 1990

GROUP B — **Veterinary drugs**(1) **and contaminants**

(1) Antibacterial substances, including sulphonomides, quinolones

(2) Other veterinary drugs

- (a) Anthelmintics
- (b) Anticoccidials, including nitroimidazoles
- (c) Carbamates and pyrethroids
- (d) Sedatives
- (e) Non-steroidal anti-inflammatory drugs (NSAIDs)
- (f) Other pharmacologically active substances

(3) Other substances and environmental contaminants

- (a) Organochlorine compounds including PcBs
- (b) Organophosphorus compounds
- (d) Chemical elements
- (d) Mycotoxins
- (e) Dyes
- (f) Others

General Method Performance Requirments

2.3.4. **Performance criteria and other requirements for chromatography coupled to infrared detection**

Adequate peaks: Adequate peaks are absorption maxima in the infrared spectrum of a calibration standard fulfilling the following requirements.

2.3.5. **Performance criteria and other requirements for the determination of an analyte using LC with other detection techniques**

2.3.7. **Performance criteria and requirements for the determination of an analyte by GC in combination with electron capture detection (ECD)**

An internal standard shall be used if a material suitable for this purpose is available. It shall preferably be a related substance with a retention time close to that of the analyte. The analyte shall elute at a retention time which is typical for the corresponding calibration standard under the same experimental conditions. The minimum acceptable retention time for an analyte shall be two times the retention time corresponding to the void volume of the column. The ratio of the retention time of the analyte to that of the internal standard, i.e. the relative retention time of the analyte, shall be the same as that of the calibration standard in the appropriate matrix, within a margin of ± 0,5 %. The nearest peak maximum in the chromatogram shall be separated from the designated analyte peak by at least one full peak width at 10 % of the maximum height of the analyte peak. For additional information, co-chromatography may be used.

General Method Performance Requirments

2.4. CONFIRMATORY METHODS FOR ELEMENTS

Confirmatory analyses for chemical elements shall be based on the concept of unequivocal identification and accurate as well as precise quantification by means of physical-chemical properties unique to the chemical element at hand (e.g. element characteristic wavelength of emitted or absorbed radiation, atomic mass) at the level of interest.

The following methods or combinations of methods are considered suitable for the identification of chemical elements:

Table 7

Suitable confirmatory methods for chemical elements

Technique	Measured parameter
Differential pulse anodic stripping voltametry	Electric signal
Atomic absorption spectrometry	
Flame	Absorption wavelength
Hydride generation	Absorption wavelength
Cold vapour	Absorption wavelength
Electrothermal atomisation (graphite furnace)	Absorption wavelength
Atomic emission spectrometry	
Inductively coupled plasma	Emission wavelength
Mass spectrometry	
Inductively coupled plasma	Mass-to-charge-ratio